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MICROSCOPY

PROBOSCIS OF BLOW-FLY. A TEST-OBJECT FOR LOW POWERS.
Photographed with a 24-mm. Holoscopic Objective N.A. ·24 and two Green Screens × 60.

[*Frontispiece.*

Physics.
Optics.
S.

MICROSCOPY

THE CONSTRUCTION, THEORY AND USE OF THE MICROSCOPE

BY EDMUND J. SPITTA

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Past President of the Quekett Microscopical Club, Author of *Photomicrography*, and Joint Author of *An Atlas of Bacteriology*

With 83 Half-tone Reproductions from Original Negatives and 255 Text Illustrations

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DEDICATED

TO THE COUNCIL AND MEMBERS

OF THE

QUEKETT MICROSCOPICAL

CLUB

PREFACE TO THE THIRD EDITION

In this third edition of Microscopy the text has been revised, and a few difficult paragraphs rewritten, in the hopes of making their meaning more easily understood. Some additional test-objects are also suggested for objectives of different focal length, illustrations of them all being either sandwiched between the original photographs, or placed by themselves in the new Plates—of which there are twelve in number—at the end of the volume.

The difficulty experienced by the student in recognising when he has obtained the often talked about "critical image" has been pointed out to me as a serious neglect in all text-books. I have therefore in the present issue attempted to meet this omission by adding a plate containing two reproductions of diatoms commonly met with, one taken with a medium, and the other with a high-power objective. It is hoped that the beginner will now be able to quickly and efficiently learn for himself, by comparing what he sees in his microscope with the photograph before him in the plate, how a critical image should appear, what it looks like—due allowance being made for a small amount of falling off in perfection inseparably connected with reproduction processes of all kinds.

Further, an explanation is given how to test and how to use a comparatively new type of low-power combination, expressly designed to meet the difficulty—much pronounced during the war—of studying and reproducing objects such as whole Flies,

Fleas, Lice, or such-like creatures of large dimensions, at different magnifications, with suggested specimens to employ. It has always been a difficulty to do this hitherto, owing either to the existing combinations of long focal length not being able to deal effectively with fields of such extended areas, or on account of the restriction imposed by the diameter of the tube of the microscope, or even it may be added when an ocular is used, to the limiting nature of its self-contained diaphragm. Several illustrations of the performance effected by these lenses —which are used without an ocular—are added in the new plates, and it is hoped they show the equality of definition produced by objectives of English manufacture, as compared with those of foreign construction.

Although, owing to the war, opticians have not been able to introduce much that is new, still in the Addenda mention is made of a few fresh objectives, besides several other matters of general interest. I should like to call special attention to the remarks concerning the use of some new short wave-length gelatine films (brought out by Kodak, Limited, in conjunction with myself) to assist resolution when used in company with high-power illuminants, both the new illuminants as well as the screens being fully described.

Whether to delete entirely from this issue, or leave intact all references to, and illustrations of, microscopes and microscopical apparatus constructed in enemy countries has been the subject of considerable deliberation. It has been thought, however, that, seeing a book of the nature of MICROSCOPY may often be consulted as a work of reference—especially by those already possessing microscopes and objectives of such manufacture—it would be better, without making any further additions, to allow the text of the previous edition to remain unaltered in this respect.

The Index has been increased, numerous new entries having been made for the convenience of quick reference.

I have again to thank Prof. A. E. Conrady for kindly giving

me his advice upon any recondite matters concerning which I felt the need of his expert assistance, and for reviewing his two chapters at the end of the volume.

My thanks too must not be omitted to several friends, notably, Commander A. Ainslie, R.N., Dr. G. Burton-Brown, Mr. J. B. Groom, Mr. D. E. Seale, U.S.A., and others, for their kind suggestions made from time to time upon various details connected with the book, all of which have been taken notice of so far as it lay in my power.

This Preface would be incomplete, however, without the acknowledgment of my indebtedness to Mr. John Murray and to his staff for their general assistance and supervision, to Mr. Emery Walker for his dexterous reproduction of the new photographs, and to the printers, who have paid such careful attention to the text as well as to the execution of the Plates, for without their joint support I feel I should have been unable to overcome the difficulties of the times.

In conclusion, I am regretfully bound to add that it has been found necessary to raise the price of this edition above that of the first and second. The cost of every item of book production is now almost three times as great as it was when the second edition was printed. Indeed, it is for the avoidance of all unnecessary expenditure that I have been compelled to place a certain amount of the new matter in a chapter at the end of the volume, which I have called the Addenda, instead of incorporating such paragraphs in the general text. Still, notwithstanding this somewhat objectionable arrangement which the exigency of the times has demanded, it is hoped that the good friends who have previously so liberally advanced the interest of the book—for which I sincerely thank them—will recognise the difficulties of the situation, and continue to offer the author their lively assistance in the future, as they have in the past.

HOVE, 1919.

PREFACE TO THE SECOND EDITION

OWING to the kind reception of MICROSCOPY, both by reviewers and by microscopists in general, whether in England or abroad, it has been found necessary to issue a second edition.

Science advances nowadays at such a pace that, notwithstanding it is but seventeen months since the original publication of this work, it has been found obligatory to incorporate in the text of the new volume a considerable amount of fresh matter, occupying some thirty additional pages. These contain information of a very varied character embracing such different subjects as: The extension of dark-ground illumination to the use of high powers; Dr. Siedentopf's arrangements for viewing ultra-microscopical particles; certain improvements in the manufacture and adaptation of microscopical stands; new objectives, and those constructed with unusually long working-distances for special purposes; original arrangements for obtaining ordinary and monochromatic light, new accessories for the microscope, and so forth.

The theoretical section, too, has not remained unaltered, for Mr. Conrady, F.R.A.S., has very kindly contributed some valuable additional remarks to his original chapters, which he considered necessary to bring this interesting and far-reaching portion of the subject completely up to date.

Although the number of the plates remains unchanged, five additional half-tone blocks are interpolated, by which it is hoped a little gap will be filled that originally existed.

It is my wish to cordially acknowledge my indebtedness to Mr John Murray, Messrs. Hazell, Watson & Viney, and all concerned in the publishing and printing of this new volume for their loyal co-operation and hearty assistance ; and not least because last, to thank sincerely Mr. A. E. Conrady, several personal friends and others, some of whom are entire strangers to me, for their congenial criticisms and valuable suggestions, which I recognise to have been of no little service and support.

EDMUND J. SPITTA.

Hove, Sussex.

PREFACE TO THE FIRST EDITION

THIS work, which I bring before the reader with considerable diffidence, is the outcome of a wish expressed by several friends that I should make the attempt to write a book upon the Construction, Theory, and Use of the Microscope expressed in the simple language employed in *Photomicrography*.

Two things seemed to encourage me : one was the gratifying way in which the last-mentioned brochure was dealt with by the critic, whose kindness in overlooking its many faults and failings had greatly impressed me ; whilst the other was the fact—which gave me no small pleasure—that so many workers with camera and microscope had expressed themselves with such pronounced generosity as to the utility of its subject-matter.

It was further pointed out, as an additional incentive to start upon the work, that a practical book on the Microscope, besides assisting the amateur, might meet a want much felt in the medical and other laboratories, where at present the lecturers and demonstrators have to waste much of their valuable time in teaching students the rudiments of the microscope by word of mouth. It is universally recognised that this knowledge could be acquired just as well from a book if a suitable one existed. Moreover, some went so far as to say that such a handbook might be of even greater service still to those who, in a later period of life, took to

the Microscope as a source of pleasure and profit, or became engaged in original research, but had not received any previous training in the scientific use of the instrument; such persons might probably be desirous of availing themselves of any help or guidance that would lead them to use their microscope to the greatest possible advantage.

To meet these requirements, then, has been the aim of MICROSCOPY.

As it is desired this work should deal with the subject from beginning to end, it was thought advisable to devote a small space to explaining the general properties possessed by lenses in general, particularising their employment as hand-magnifiers or as hand-microscopes, whilst to aid more advanced students a full description of the method of testing objectives and condensers has been added. For this purpose special attention has been paid to the use of the Abbe test-plate, which I believe is not dealt with in any text-book in the English language.

But, to enable the reader to follow the subject from beginning to end intelligently, it has been found necessary to interpolate articles upon what might be called the more recondite problems connected with microscopy, such as the estimation of the magnifying powers of objectives and oculars by the "rational" method, as well as by the system devised by the late Prof. Abbe; the explanation of what is really meant by the expression Numerical Aperture; upon the art of obtaining and using Oblique Light, with the theory involved in so doing; the importance of the proper use of the Substage Diaphragm; the selection and special adaptability of objectives of certain focal length and numerical aperture for particular purposes, as well as an explanation of the real difference existing between the semi-apochromatic and apochromatic combinations. Further, as the Microscope is nowadays the handmaid of so many of the Arts and Sciences, it seemed absolutely imperative to devote a chapter to the assistance

of those about to embark upon particular branches of the subject, showing what are the special requirements attending each case; for it is very obvious the kind of apparatus demanded, say, by the bacteriological student, would be of an entirely different character from that required by one whose aim was the investigation of the strain in metals, or the details of the molecular arrangements in various kinds of iron and steel; let alone the special wants of the student of simple pond life or botany, in comparison with one whose aim was the discovery of the final structure of diatoms.

Lastly, in consequence of the principles which underlie the formation of the highly magnified microscopical image, more especially when the instrument is dealing with minute objects of periodic structure so small as to be commensurate with the wave-lengths of light, I have thought it would be an omission on my part if no mention were made of so engrossing and far-reaching a topic. Seeing, however, that the explanation of a difficult problem of this nature, to meet the requirements of the strictly philosophical student, necessarily involved its careful consideration from a purely theoretical standpoint, I have availed myself of the kind assistance of my friend Mr. A. E. Conrady, F.R.A.S., as I felt that his intimate acquaintance with the subject in all its mathematical intricacies would enable him to grapple with and explain it in a far more scientific and philosophical manner than I could hope to do myself.

I have to thank the opticians in many parts of the world for having kindly lent me blocks of their manufactures, besides permitting me to examine critically their objectives; as well as the Scientific Press—the proprietors of my book on *Photomicrography*—for their permission to use a few excerpts, blocks, or diagrams taken with this distinct acknowledgment from that work. Also I am greatly indebted to Mr. Alfred Dent, of the firm of Dent & Co., for the personal attention he has given to the manufacture of the extra blocks herein

contained over and above the few kindly lent by the Scientific Press, to which reference has been made; to Messrs. Hazell, Watson & Viney and their executive for the care and trouble they have taken not only throughout the work, but especially in *printing* the Plates so as to obtain as much from the blocks as possible; as well as to Mr. John Murray for sparing no expense incurred in this matter.

Two debts of gratitude yet remain unacknowledged; one is to my friend Mr. Conrady, not only for his chapters above mentioned, but also for the trouble he has taken in assisting me with numerous details, as well as for his kindness in reading through a considerable portion of the MS. before its final issue to the Press; and, lastly, to the Council of the Quekett Microscopical Club in granting me their permission to dedicate this little effort to the "Club."

In conclusion I have only to express the hope that, in reading MICROSCOPY, the critic will be kind enough to overlook its many failings, bearing in mind that it is not intended to compete with the highly classical and standard works already written upon the subject, but merely to represent "a special effort to meet a special end."

EDMUND J. SPITTA.

HOVE, 1907.

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THE MICROSCOPE

CHAPTER I

INTRODUCTORY

THE word Microscope, derived from two Greek words *μικρός* (small) and *σκοπέω* (to see), is the name of an instrument that magnifies an object.

A microscope may be of two kinds—simple or compound. The simple consists of a single "lens" or several "lenses" mounted rather close to each other in a suitable handle, and is intended to be used for obtaining magnified erect images of small objects in the way familiar to everybody possessing an ordinary pocket "lens." The compound microscope consists of two widely separated "lenses" or sets of "lenses," one of which, called the objective, serves to form an inverted image of the object to be examined, this image being further magnified by the second set, called the ocular or eyepiece. For convenience in use the objective and eyepiece are held at the opposite ends of a tube which, in its turn, is attached to a "stand."

The word "lens" above used calls for an immediate explanation, hence it will be advisable before proceeding further to explain the meaning of the term and to name and describe the several varieties.

A lens is the name given to a piece of glass or other transparent medium—usually circular in form—having its two faces ground and polished in a specific manner, which imparts to it the property of causing rays of light from a distant point after passing through to converge together or diverge apart. The curves on these faces may be spherical, cylindrical, or of

a parabolic nature, but it is only with the first type that the microscopist has to deal. The combination of a spherical surface on one side with another on the other, or with a face that is plane, gives rise to eight different kinds of lenses. These are shown in Fig. 1 The first is a double or bi-convex, because the convex curve on each side is similar; the second is a plano-convex, one side being plane and the other convex; whilst the third, having a convex surface on one side and a concave on the other—the former of which is the more pronounced—is called a convexo-concave or converging meniscus. The fourth, with both sides concave, is termed a double or bi-concave; the fifth is plano-concave; the sixth a diverging meniscus or concavo-convex, because the ruling curve is

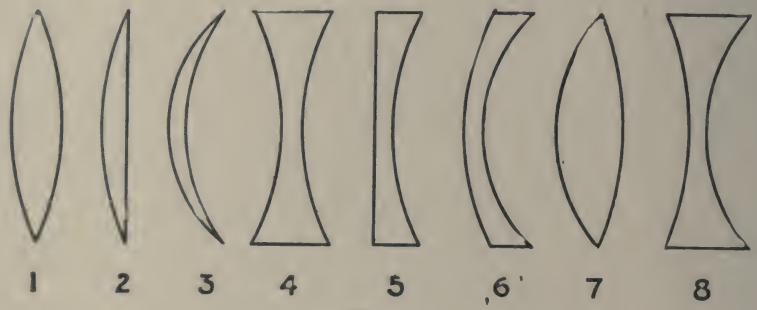


Fig. 1.

a concave one; the seventh a crossed convex, because although both surfaces are convex they are of different curvature; and the eighth a crossed concave, as each surface is concave although of dissimilar curvature. It should be noticed that numbers 1, 2, 3 and 7 are all thicker at their centres, whereas 4, 5, 6 and 8 are all thinner. The former are all termed in a general way convex, positive, or collective lenses, as they will be shown to gather and converge rays of light from a distant object to form a real image of the object, *i.e.* one which can be received on a screen; whereas the latter are collectively known as concave, negative, or diverging lenses, because rays falling upon them are diverged or dispersed and so do not form real images. In the description that follows the double convex and the double concave are only considered, as the properties possessed by these two types apply equally to

the remainder, which differ from them only by the degree and nature of certain defects called aberrations. It is also assumed that the thickness of the lenses is so small as to be negligible.

The curves of a lens are described from given centres, each being called the "centre of curvature," and the *radius* of such circle, of which the curve is an arc, is called the "radius of curvature." The line joining such centres passing through the body of the lens is called its axis, or the "principal axis of the lens."

Before explaining the passage of light-rays through different lenses, showing how in one case the beams are bent differently from those in another, it will be necessary for the context to be intelligibly followed to make a few introductory remarks upon the subject of light in general, and upon refraction in particular; but the reader must not expect in what immediately follows to find the matter treated in a rigidly mathematical manner, for that is given in another part of this work, but rather in as simple a way as possible.

The rays issuing from an illuminant, assumed to be of small size, naturally *di*verge in all directions from that point; but if we consider the light falling upon a comparatively small object from a very distant illuminant such as the sun, the *di*vergence is so infinitesimal that we may safely regard such a light as consisting of parallel rays. It may just be remarked here, that *con*verging light does not exist in nature: it can only be obtained by refraction or reflection, and to some extent by diffraction, but this matter will be referred to later on. The path of any ray in a given medium is always straight until the beam or pencil meets with another medium more or less dense, when, with one exception noted hereafter, it is bent aside, undergoing what is called refraction; after such bending, however, it will again resume its rectilinear propagation along its new path, until it meets with a fresh medium, when on entrance it will be bent again if the density of such medium be different. Change of density, then, *of the medium* is the cause of refraction, and this should be held in mind. It will be of advantage now to point out briefly the nature of this alteration of direction brought about by the change of medium, and for purposes of description to have to resort to Fig. 2.

Let ABCD be a vessel containing water and AC the water

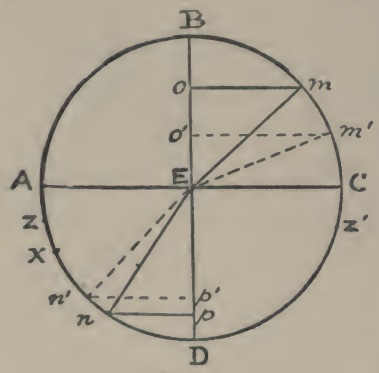


Fig. 2.

line; BD, the Normal, is drawn at right angles to AC, cutting it at E. All angles are referred to this line. When the beam is incident along B and E perpendicularly into the new medium there is no refraction—the only instance referred to when it undergoes no bending—for it passes on into the water, uninterruptedly following the course of the line ED. But when it is incident at any other position—say at *m* along *m*E—there *is* refraction at E, for the beam will be found to strike the point *n*. Suppose it is incident at *m'* along *m'*E, then there is also refraction at E, for the ray will be found at *n'*.

Snell made a celebrated investigation concerning this bending of the rays, by which their path can always be predicted.

He first drew a line from *m* to meet BE at right angles at *o*, and another from *n* meeting ED at *p*. The lengths *om* and *np* were measured and divided, the greater by the less, and a quotient obtained. Now what he discovered was the fact that, wherever the angles were taken, whether from *m* or *m'*, the quotients—in all cases using air and water—came out the same, viz. 1·33. This he called *the refractive index of water*. Other substances were substituted for the water, and each substance he found had its special refractive index. Flint glass, for example, was found to be about 1·54 to 1·64, according to its manufacture, and so on with the other substances, complete lists being found in all books upon the subject. If the reader be mathematically inclined, he will at once see these lines, *mo*, *np*, really represent the sines of the angles BE*m* and *n*F*p* respectively, so that, continuing our precept, the sines bear a certain definite ratio one with the other wherever the incident light striking E may come from[1]; that is, the ratio between *om* and *np*, which is about as 4 is to 3, holds good, whether the

[1] In other language, the sine of the angle of incidence divided by the sine of the angle of refraction is a constant quantity. It will be seen later, on page 92, that refractive index is the ratio of the velocity of light in air, to that in a given substance.

ray starts from *m* or *m'*. It is quite evident now that we can calculate where the ray will strike the arc AD, after starting from any given point in BC. For example, let *m* strike E to make an angle *m*E*o*, say, of 45°. It is required to find the angle *n*E*p*, so that we can draw *n*E correctly. We take out of the ordinary tables the natural sine of 45°, and find, roughly speaking, it is 0·7, and multiplying that by 3 and dividing by 4 gives us 0·5. Resorting once more to our tables, we find 0·5 is the sine of 30°, so that 30° must be marked off from D to find the position of the line *n*E. Although simply put, this is the idea that mainly pervades the computer's mind in constructing new lenses. As a matter of fact, the details become exceedingly operose in real calculations, as different colours are refracted at different angles; and so the problem, where many lenses are concerned, becomes intensely intricate. But the law underlying these calculations is the same from beginning to end. The same law—reversed, of course—holds good when rays pass from the water into air, and when passing from one kind of glass to another, although then with certain modifications which need not here be mentioned.

One more remark. Seeing that *n'*, passing to E, becomes refracted to *m'*, what will happen to a ray starting, say, at *x*? It will pass into the air and graze along EC. If this be true, what will take place if one starts still nearer A, say at *z*? This ray cannot get out of the water at all, and so is said to suffer "total reflection" at E, for it appears again at Z'. There is one angle, then, it is very evident, which is the last that allows a ray to get out; this is called the "critical" or "limiting angle."

We have seen, then, the first point to notice is that the path of the ray is largely affected by the density of the glass employed in the manufacture of the lens.[1]

If a ray passes in the direction of the normal upon the surface of a piece of glass having parallel sides, it continues its course uninterruptedly just as it did in the case of the water; but if the ray be incident at any angle—say as in Fig. 3—it is bent by the glass *towards* the normal NN, because it is entering a medium that is denser than air. The amount of bending of course varies with the refractive index of the vitreous compound. But the

[1] The substance of this paragraph and the diagram are taken from the author's *Photomicrography*, with the kind permission of the Scientific Press.

emerging beam on leaving the glass is bent this time *away from* the normal, because it is entering the air, a medium less dense. As it quits the glass then to re-enter the air, the same medium in which it started, so it is bent back again the same amount in the opposite direction as it was deviated in the glass; hence the incident and emerging rays are parallel, although not in direct continuation one with the other in a straight line. If, instead of emerging into the air, it had entered another piece of glass, or other medium of *different* density, its direction would have been changed directly in accordance with the change of index of refraction of the new medium.

When, however, the glass has not parallel sides, but is in the

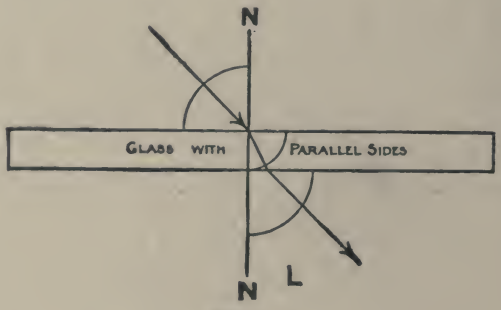


Fig. 3.

form of what is called a prism, the conditions being modified, the results are not similar.

A prism may be defined optically as a transparent medium contained between limiting surfaces which meet together at one end, forming what is called the *summit* of the prism, the separated ends below being joined together by a boundary called the *base* of the prism. The angle enclosed by the two sides that meet to form the summit is termed the *refractive angle.*

Fig. 4 shows three prisms, and the summit, base, and refractive angle of each. When the refractive index of the medium composing the prism is known, and the normal drawn, the path of the ray can be struck as previously shown. Let ABC in Fig. 5 be a prism, OD the incident beam, and the dotted lines at D and K normals to the respective surfaces with which they are in contact. The incident ray OD, on entering the glass, is

bent towards the normal, because it is entering a denser medium, its path being shown as DK. On leaving the surface AC, it is bent away from the normal in the direction KH, because it is entering a medium of rarer density. If the eye be placed at H, the ray appears to come from O′, so the object O appears in that situation. It should be noticed that in this case the ray

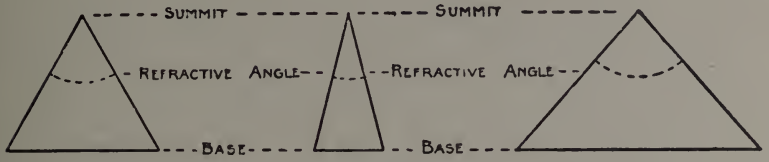


Fig. 4.

has been twice bent *in the same direction*, so that it is turned towards the base of the prism ; hence it should be borne in mind that objects seen through a prism appear *shifted towards its summit.*

The angle O′EO is called the angle of deviation.

As a matter of fact, objects under these conditions are seen in all colours of the rainbow, constituting the phenomenon called the *dispersion of light* ; but of this subject we speak no

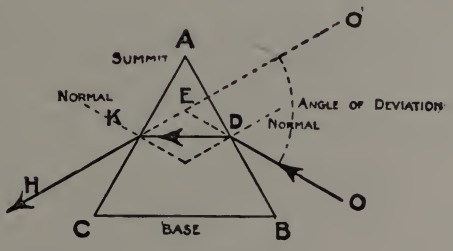


Fig. 5.

more at present, although its effect is discussed later, when the construction of objectives for the compound microscope is being dealt with.

In discovering the path of the rays passing through different portions of a *lens*, it is legitimate to consider such as being made up of a series of superimposed *prisms*, as shown in Fig. 6, bearing in mind that, with a positive lens, the summit of each prism is directed towards the periphery of the lens ; but in the

case of a dispersive or negative one, the summit is turned in the opposite direction : in other words, *the base* is turned towards the periphery. When computing the exact path of the ray it is of course necessary to consider the number of these prisms as infinite, and only consisting theoretically of the actual strip of glass occupied by the ray in question. Each little prism may also be said to possess its plane surface coinciding w.th and constituting the lens surface itself—both being one and the same thing—so that the curved lens front may be analytically considered as consisting of an aggregation of an infinite number of plane surfaces of infinitely small size. A line drawn perpendicular to any of these theoretical planes is called its Normal. But little further consideration will suffice now to show that all

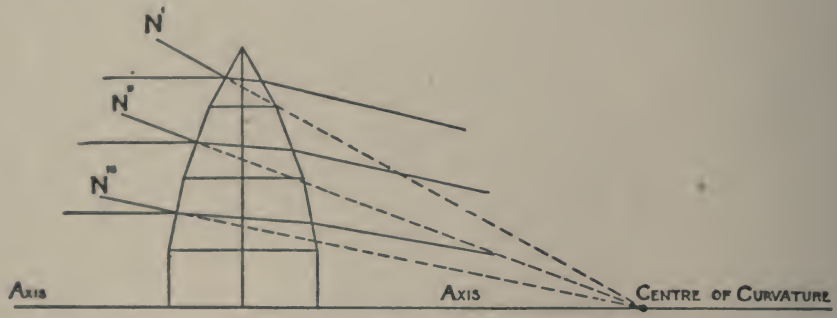


Fig. 6.

or any of these normals, N′, N″, N‴, in Fig. 6, for example, when produced will take the path of the *radius* of curvature, and so arrive together to meet at what is called the *centre* of curvature.

Collective lenses, it has been stated, converge the rays falling upon one side to form an image of a sufficiently distant object on the other (Fig. 7). This position where the image is formed is called the "plane of focus." When the object is very distant and the rays from it practically parallel, the focal plane resulting is called the *principal* focal plane, and its distance from the lens the *principal* focal length.

The act of making the rays meet to form the image on the suitably placed screen is known as "focussing the object," and the point of union of the rays "the focus."

Negative lenses, we have already stated, are dispersive, and so cannot form a "real" image of an object on a screen; hence it may seem very illogical to at once state they are differentiated one from the other and designated by the same nomenclature as positive lenses which *do* form real images. But the phrase "focal length of a negative lens" has a conventional meaning, for it is intended to imply that such a lens—say we speak of "a 2-in. negative"—has the property of *neutralising* the collective power of a *2-in. positive one.*[1] If, therefore, a 2-in. negative and a 2-in. positive be placed in juxtaposition, and an object looked at through them, it is neither magnified nor diminished, for the two lenses act just the same as if they were one piece of glass with parallel sides. This fact is taken advantage of by the practical optician. If he has an unknown

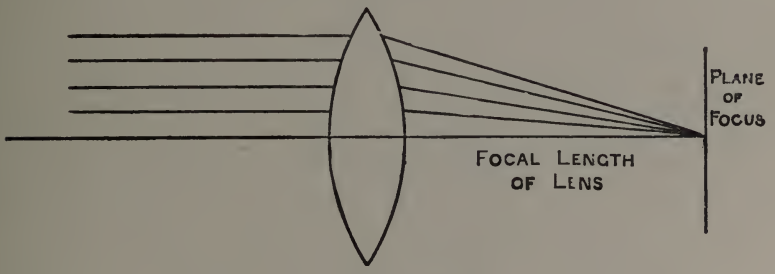


Fig. 7.

negative lens, and he desires to ascertain its focal length, he keeps trying several positives with it until, when looking through both lenses at an object, such object ceases to "shake," as it is called, when he moves the lenses. When this result is obtained, he knows he has neutralised the negative, and the *focal length of the positive lens* necessary for this purpose is called the *focal length of the negative.* The same can be done with an unknown positive by using a set of negatives of known focal lengths.

In describing the rays entering and leaving a lens it is convenient to know which are being spoken of. Rays falling *upon* a lens are called *incident* or *affluent*, while those *quitting it* are

[1] Really, the same definition covers foci of positive as well as negative lenses, if a "virtual" focus be accepted for the concave. The term "virtual" is explained hereafter.

termed *emergent* or *effluent*. Rays that become closer and closer together as they advance are called "converging," and those which separate further and further apart are designated "diverging." Rays that run side by side without diverging or converging are called "parallel."

With respect to a convex lens, when incident rays from a pencil further away than its principal focus have traversed it, they emerge convergent. But when rays have passed through a concave lens they leave it divergent, or—in the case of light entering strongly convergent—*with diminished* convergence.

The emerging rays from a convex lens converge, in most instances, it has been said, to form an image, but as the distance from the lens at which this image is formed varies with the position of the object—or radiant or luminant, as it is sometimes

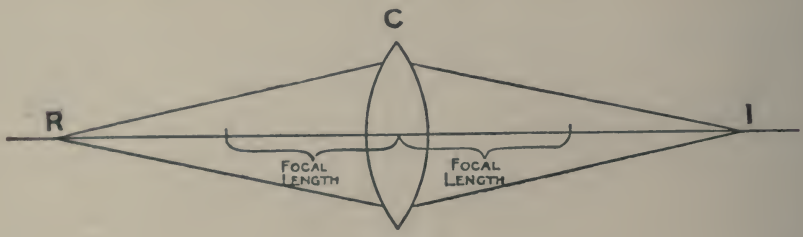


Fig. 8.

called—it will be well to explain in detail the relation which exists between the object and its image.

If a radiant R be placed at a given distance—for convenience of description let it be said in the first instance now under consideration to be equal to twice the focal length of the lens—an image will be formed on the other side of the lens at twice its focal length. This is shown in Fig. 8, where the distance of R to the lens equals that from I. The distance, then, from R to I is equal to four times the focal length of the lens. If R be now moved further away from the lens C, as in Fig. 9, I is situated nearer to it. If R be moved still further away, I draws nearer still to C than before, and so on until R be placed so far away that the rays coming from it may be considered parallel as in Fig. 10. The position now occupied by I *under these conditions* is said to be that of *lying in the principal focus* of the lens. Because it is not possible to place

R at a sufficient distance for the rays to be *truly* parallel, so the sun or the moon or the stars are usually selected, as *their* rays may be called sensibly parallel: hence the principal focus of a lens is often spoken of as "the solar focus." Seeing the positions of R and I are theoretically interchangeable, if the radiant be placed at the focus I the rays issue parallel on the

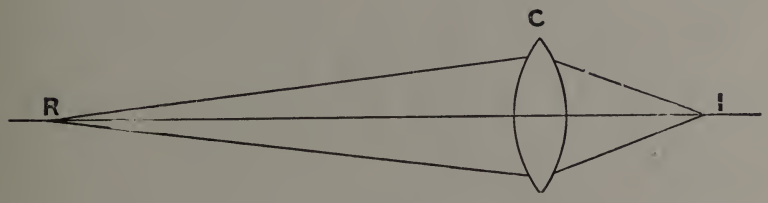


Fig. 9.

other side. Hence a rule may be stated, "*That if a radiant be placed in the 'principal focus' of a lens, parallel rays issue from it on the other side.*" But in the preceding diagrams, Figs. 8 and 9, where neither rays are parallel, and seeing that the radiant and image are interchangeable, the focal lengths bear a certain relation one to the other, for as one increases the

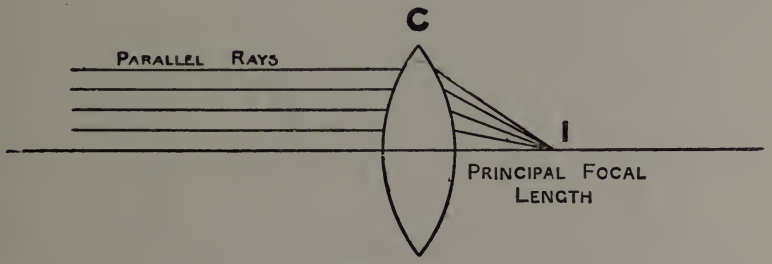


Fig. 10.

other diminishes, and *vice versâ*; hence R and I are spoken of as conjugate foci.[1]

[1] Although hardly within the scope of a work on microscopy, still it may be at times desirable to ascertain the relative positions of the conjugate foci, by which is meant, If an object be placed at a *given* distance from the lens on one side, where is the image formed on the other side?

This is effected by solving the simple equation—

$$\frac{1}{V} = \frac{1}{U} - \frac{1}{f},$$

We have now discussed how a convex lens deals with rays that are *di*verging, and also the effect it produces on those from a radiant placed at so great a distance that the individual beams are *parallel*, as in the case of sunlight. It remains yet to be shown what effect it has upon beams that are *con*vergent upon it.

Such a condition is not met with in nature, but it may be in the formation of certain lens-systems where several lenses are joined more or less nearly together. Such a condition is set forth in Fig. 11, where rays are shown falling upon C, having been cast there by the auxiliary lens C′. This lens

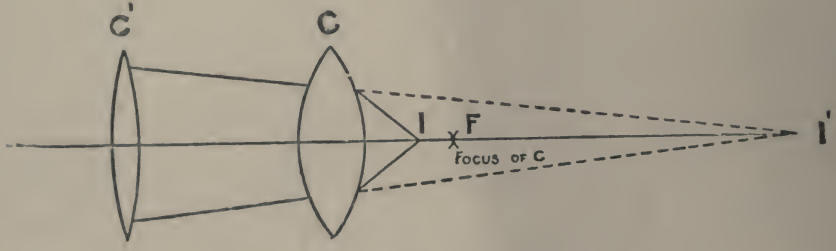


Fig. 11.

would have focussed the rays at I′ if left alone, but the interposition of C shortens the focal length, so that the focus now takes place at I instead. C has therefore *a shorter focal length than would occur had the rays been parallel that fell upon it*, for they would of course have focussed at F. This change, it should be understood, is entirely brought about by

where V is the distance required, U the distance of the object from the lens, and *f* the principal focal length.

Take the following example which might occur in microscopical considerations:

Let U equal ·204, *f* equal ·2 in., when V will be found to be $-10\frac{1}{2}$ in. Hence the conjugate focus is easily found by taking $10\frac{1}{2}$ in. from the lens on the other side.

It should be mentioned that when U is greater than *f*, V is *negative* (as in this case), so the image lies on the *opposite* side of the lens to that of the object; when, however, V is less than *f*, V is *positive*, so the image is on the *same side* as the object.

The magnification, it may be stated, of the image as compared with the object is as V is to U—hence in the above case the amplification is 50 diameters.

the convergency of the beams in question. Hence as a rule to recollect, "*When converging rays fall upon a convex lens it focusses them at a plane which lies within its principal focus.*"

We may further just remind the reader, if a radiant were placed at I (within the true focus) the rays issuing from C would be *di*vergent instead of parallel, as would obtain had it been placed at F, the principal focus.

It can readily be inferred from what has been said how great changes can be effected in the path of rays by combinations of lenses associated in this manner, and moreover how such can be additionally modified by the distance the components are apart. It is on this account, in the manufacturing of lens-systems, the optician has to be so careful all the components shall occupy precisely their exact relative position one with the other that are set forth in the computer's formula.

The following five principles may now be tabulated:

1. When parallel rays enter a collective lens they are united in a focus at a certain distance from it on the other side, such distance being called the principal focal length of the lens.

2. Conversely, if a radiant be placed at the principal focus of a lens, parallel light issues on the other side.

3. Rays falling upon a collective lens in a *di*verging manner, within certain limits leave it in a converging manner to form a focus of the radiant.

4. Rays falling in *convergency* leave it with greater convergency still, to form a focus which lies within the principal focus.

5. If a radiant be placed *within* the principal focus of a lens the rays leave it in a *diverging* manner, although to a less degree than those incident upon it.

So far the radiant has been mostly depicted as a point of light and usually situated on the axis of the lens; it remains now to be shown what happens when the object is one of sensible area. Not much difficulty should be here experienced when it is recollected that a self-luminous object of sensible size is nothing but an aggregation of self-luminous points. There is this which is new, however: *these points in this case are not all situated on the axis of the lens*, hence their paths should be indicated to clear the reader's mind. Let Fig. 12 be considered, the arrow BC being an object of sensible size

and AA′ the axis of the lens L. The rays from B of course radiate in all directions as from any other point, and those that fall upon and are collected by L pass through it, being bent to focus and form an image at *b* as indicated in the figure. Those from C focus at *c* to form that part of the arrow, whilst the rays issuing from other points of BC are cast (although not shown in the diagram for clearness of rendering) in their respective positions to fill up the gap between *b* and *c*. It is obvious now that the image becomes inverted as compared with the object. If the object be further from the lens than its image, the latter is smaller than the object, whilst if the former be nearer than its image, the latter is greater. This is easily understood as the natural results of the working of the conjugates. It should be noted for convenience of future

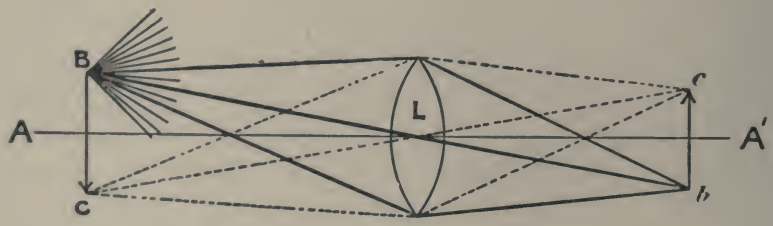


Fig 12.

reference that the entire system of rays issuing from, say, B or C, or both, are not always shown in a diagram because of complicating it, so that it is not uncommon to represent the whole mass of rays by simply drawing their axes, such as B*b* or C*c*, which materially lessens the number of lines in a figure and yet furnishes all that is really required. Such axes are called "the secondary axes of the lens." The matter is merely referred to here as such figures have often been known to puzzle considerably the beginner unacquainted with the subject.

To ascertain the principal focal length of a lens, it is only necessary to throw the image of the sun (which means the use of parallel rays) upon a screen suitably placed, and to measure the distance of such image plane from the lens. As, however, it is not easy at all times to locate, for the purpose of such measurement, the point of the lens to measure to, it may be

necessary to adopt another method. It is based on the fact already mentioned that if an object be focussed on a screen by the lens in question so that the *image* shall have exactly the same size as the *original*, the distance between object and image is exactly four times the principal focal length, so that

Fig. 13.

AB, BL, LC, and CD are all equal, and BL and LC equal to the principal focal length of lens, therefore each is one-quarter length AD, so AD divided by 4 equals the principal focal length of lens. This can only be accomplished if the image A is exactly the size of the object D.

dividing this distance by four furnishes an accurate value.[1] See Fig. 13.

Occasions, however, may occur when it is desired to learn hastily, *approximately anyhow*, the principal focal length of a convex lens, the sun or moon perhaps being absent, and the means for carrying out the equalisation of image and object not at hand. The following is then useful. A radiant is

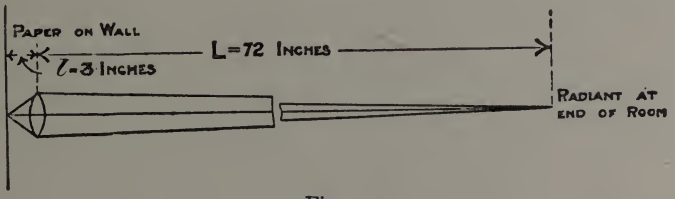


Fig. 14.

placed at the end of the apartment and its image cast on a piece of paper pinned to the wall at the other and opposite end, as in Fig. 14. The exact distance between paper and lens is taken, and between radiant and lens also. Let L equal to

[1] Although we have stated this positively, still it should be borne in mind that the distance between object and image is *not* mathematically four times the principal focal length; it differs from that by the distance between the two cardinal points. For further information the reader is referred to text-books upon Optics, as it would seem beyond the scope of this work to enter into the subject at greater length. See also Appendix.

72 in. be the latter, and l equal to 3 in. the former. The principal focal length f then can be found by solving the simple formula following :

$$f = l - \frac{l^2}{L} = 3 - \frac{9}{72} = 3 - \frac{1}{8} = 2\frac{7}{8} \text{ in.}$$

It is obvious why this reduction of l is necessary, when it is recollected that as one conjugate lessens, the other increases ; hence as the rays coming from the end of the room are not parallel so the conjugate on the other side must necessarily be a little longer than the principal focal length ; this the formula rectifies by showing how much it has to be reduced.[1]

Sometimes, however, it is not easy to ascertain the distance of the object from the lens. In this case the differences in magnification of image and object are utilised in the following manner. An illuminated glass scale is placed just beyond the principal focus F of the lens on one side, and a screen on the other, in such a position as to receive an image of a portion of the scale greatly magnified.

Let l be the length of a given part of the scale, L the length of the same portion as seen on the screen in the magnified image, and d the distance of the screen from the lens. Then the principal focal length is found by solving the following :

$$f = d \frac{l}{l + L}.$$

There is yet another method of obtaining the focal length, and that is by the use of a collimator. This is an instrument constructed to produce parallel rays artificially. An object glass is fixed at one end of a tube, and a piece of ground glass at the other, in such a position that the sun is sharply focussed upon it. The ground glass removed, its position is occupied by a slit of very small dimensions in a piece of brass, which otherwise covers up this end of the tube. If, now, a light be placed immediately behind the slit, parallel rays will issue from the lens, as the radiant has been placed at the principal focus on the other side. With these rays the principal focus of the

[1] This formula is very closely accurate if the principal focal length is *short* compared to the available distance ; if however it be long, then the absolutely rigorous expression $f = \frac{l\ L}{L + l}$ had better be substituted. The difference in the above example, however, is only ·005 in.

lens can be found just in the same manner as if the radiant had been the sun, and the focal length thus obtained is therefore the principal one.

The principal focal length of a negative lens is often obtained by finding what positive it will neutralise; the principal focal length of the positive that fulfils this condition is said to be that of the negative.

Another method, especially convenient when positive lenses are not at hand, is to let sunlight fall on the negative, and ascertain at what distance it must be placed from the screen for the circle of diffused light to be twice the diameter of the lens in question. This distance is the principal focal length. A convenient plan to carry out this method is to measure first the diameter of the negative, and then draw a circle on a piece of white paper with a diameter twice as large. Now hold the negative at different distances until the diffused light exactly fills this circle: the distance fulfilling this condition is the principal focal length required.

All we have said so far refers to simple *thin* lenses only, and cannot be immediately applied to thick lenses or to combinations of several thick or separated lenses. For a long time all attempts directed towards the discovery of simple and universally applicable rules for determining the position and size of images produced by *lens-systems* proved unsuccessful; ultimately, however, the great German mathematician, Gauss, found a solution of this important problem which is as perfect and complete as it is elegant and simple. According to his method it is only necessary to determine the two principal foci of any lens-system, and its so-called equivalent focal length, in order to be able to solve all problems concerning the position and size of the image. For a brief account of this method the reader is referred to the Appendix, where sufficient will be found for the purposes of the Microscopical student.

CHAPTER II

THE SIMPLE MICROSCOPE

THE simple microscope is of two kinds, the common magnifying glass and the hand magnifier. The former usually consists of a single bi-convex, a crossed convex, or a plano-convex lens ; but in its best form it is composed of *two* glasses adjusted to correct certain aberrations inherent in all *single* lenses. The hand magnifier is of higher amplifying power, and has been brought to great perfection in recent years both by English and foreign opticians. In its best form it always consists of two, and very often of three lenses of suitably chosen glasses to secure the finest results. Its magnifying power usually varies from five to twenty diameters.

With this class of microscope it often happens that its magnification is required to be known. Although such information is usually afforded by the optician, still it may be desirable for the user of the lens to be able to verify the same for himself. This may easily be done in the following manner :

A small piece of a foot-rule—a mere fragment will do—is fixed on a suitable support, and the magnifier placed in the position that yields the sharpest image of this fragment when the eye is placed as near the lens as possible.[1] A complete foot-rule is then examined with the *naked* eye, and the distance at which it is best seen carefully noted. This distance is technically known as the observer's "distance of distinct vision." It may vary from 5 in. with a short-sighted person to 20 in. or more with a long-sighted one. The distance for the so-called normal eye is usually said to be that of 10 in. or 250 mm. The complete rule is then fixed up so that when one eye is looking *through* the magnifier at the fragment, the

[1] How to ascertain the best position theoretically at which to place the eye is given later on.

other eye sees the complete rule. Each object then may be said to be at the distance of distinct vision, one for one eye and one for the other. This is rudely shown in plan in Fig. 15. A

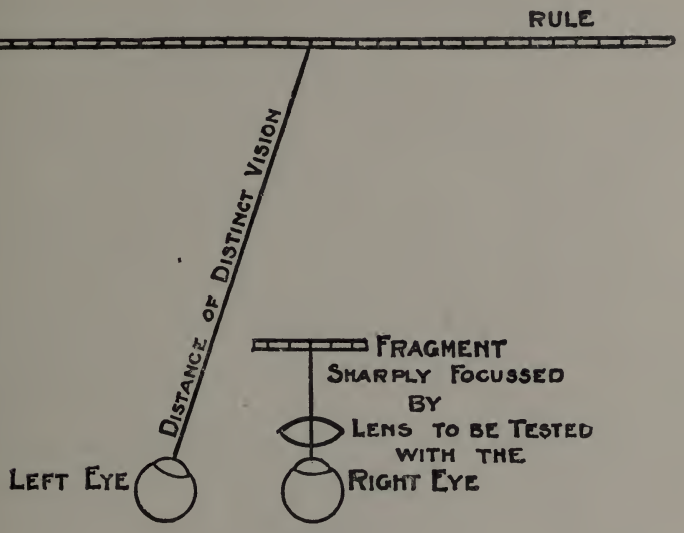


Fig. 15.

little practice is here necessary, for the mind must accustom itself to recognise *both* images superimposed one on the other at the same moment. As an example, let half an inch of the fragment be the chosen unit of measurement, using, say, the right eye, whilst the left is looking at the complete rule. The

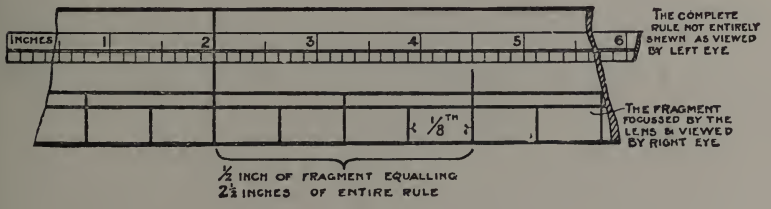


Fig. 16.

eyes and images must now be so regulated that the magnified fragment lies on the complete rule, as it is attempted to show in Fig. 16. There it will be seen that half an inch of the rule

fragment is so magnified as to apparently cover from 2 to 4½ of the scale of the complete rule, which means a magnification of 5 diameters as the half inch appears to cover 5 half inches. We say, then, that the magnifier amplifies 5 diameters. This method has only one drawback, that different results are obtained according to the observer's "distance of distinct vision"; for it is obvious, if this be at 5 instead of at 10 in., the result will be dissimilar from that of the long-sighted with a 20-in. distance of distinct vision. From this it is evident, in the evaluation of different lenses, no end of confusion would arise according to the distance of distinct vision of each observer! To avoid this, the magnifying power of all lenses is estimated by using a normal distance of distinct vision of 10 in., the short-sighted recollecting the lens will magnify less to him, and the long-sighted bearing in mind it will magnify more for him. How the difference is easily ascertained and

Fig. 17.

corrected will be given later on, for it will be convenient first to show and explain how magnification is produced in the simple microscope.

No mention hitherto has been made as to what the *eye perceives* if it be placed in the path of the emergent beam coming from a convex lens.

First let a lens focus the image of an object OO, Fig. 17, placed at a point beyond its focus F, as at SS. If the eye were placed *there*, nothing would be seen but a confused mass of light; but if the head were withdrawn to S'S'—provided the distance S'S' to SS were equal to the observer's distance of distinct vision—the eye would at once see the *inverted* image of the object, the arrow, much reduced in size, being suspended, as it were, in the air. If, however, the object be placed *within* the focus of the lens instead of *without* it, and the eye be placed *in* the emerging beam, the observer will very readily find a position close to the lens where an image of the object

appears to his eye erect and magnified, and which seems to lie on the same side of the lens as the object, although at a certain distance beyond it. In Fig. 18, let OO be the object placed within the focus F of L the lens ;[1] the rays coming from O and O are shown passing through the lens and re-issuing the other side to fall on the pupil of the observer's eye. Tracing these backwards through the lens, as shown by the dotted lines, they reach O′ and O′, forming the image O′O′ as if in that situation which is obviously magnified and erect. The distance from the eye at which this image is *apparently* formed is that of the distance of distinct vision of the observer. The image, however, is not a real one and cannot be received on a screen, but is mental, and so would not form an image on a photographic plate or upon a screen placed in the position O′O′ as indicated. For this reason

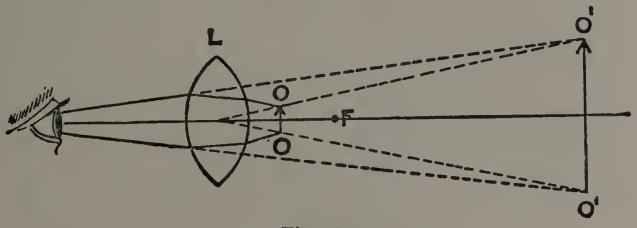


Fig. 18.

the image is called a *virtual* one, and the distance from the lens at which it appears is called the *virtual focal length.*

From what has been said the following deductions may be enunciated :

1. When an object is placed *before* a convex lens, at a point *without* the focus, an inverted image is formed on the other side of the lens by the emerging rays. Such image is real, and can be photographed or seen when cast upon a screen.

2. When the object is placed *within* the focus no *real* image is formed at all—that is to say, no image is formed that can be shown on a screen ; but if the eye be placed in the emerging beams, an image which is erect and magnified *can be seen* as if on the same side of the lens as the object, projected, as it were, at the distance of distinct vision of the observer, but which, being mental and not actual, is called *virtual*, and can only be

[1] For clearness of rendering F is placed farther away from OO than it should be.

photographed by the use of auxiliary lenses, a matter which, for the present purpose in hand, need not be considered.

These remarks have been made in as simple a form as possible, but to those who desire to enter more fully still into the subject, the following may be welcome, which is explained in somewhat different manner :

It should be first stated that the apparent magnification of

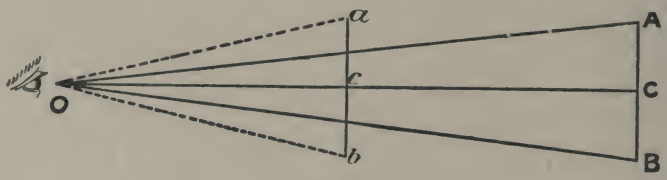


Fig. 19.

an object to the *unaided* eye depends upon the angle it subtends to the observer. Thus in Fig. 19 let AB be the object and O the observer's eye ; the apparent magnification, then, of the object is the angle AOB contained by the two visual rays drawn from the centre of the pupil to the extreme ends of the object. But if the same object be now placed at *ab*, the angle *a*O*b* is evidently greater, and so the object is said to be larger.

To the eye *which is applied to a lens*, as in a form, say, of the simple microscope, the apparent magnification of the object

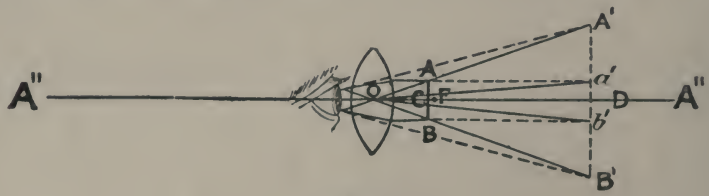


Fig. 20.

is the ratio of the diameter of the *virtual* image to that of the *real* object, both being at the distance of distinct vision as already explained. To make this quite intelligible, let Fig. 20 be considered.

AB is again the object, and A′B′ the virtual image. As A*a*′ and B*b*′ are drawn parallel to the axis A″A″, so *a*′*b*′ is equal to AB. The magnification, then, is evidently equal to the angle A′OB′ divided by the angle *a*′O*b*′, or the length of A′B′ divided

by that of $a'b'$, or A'B' divided by AB. As the angle A'OB' evidently equals AOB, so A'B' bears the same relation to AB as DO does to CO. But DO is very approximately the distance of distinct vision and CO nearly equals FO, the focal length of the lens; therefore the magnification practically equals the ratio of the distance of distinct vision to the focal length of the lens. Of course the magnification becomes *greater* as the focal length of the lens becomes *smaller*, and at the same time becomes greater as the distance of distinct vision becomes increased.[1]

From the foregoing, two more enunciations may be made:

1. That when the same object is seen at unequal distances the apparent diameters vary inversely as the distances, so that the same object at twice the distance is *seen* at half the size.

2. That in the case of two objects seen at the *same* distance the ratio of their apparent diameter is exactly similar to that of their magnifications.[2]

It has been previously mentioned, in seeking the measure of the magnification of a simple microscope, that the object should be situated within the focus in a position *that furnishes the best vision to the eye* when placed as near as possible to the lens. It was then stated that the actual position furnishing the best results could be computed. The following is the method, when desired, of so doing:

[1] A word about the angles subtended by objects might not here be out of place, for the want of a better situation for mentioning the same. It is that in using optical instruments, the angles met with and dealt with are usually so small the arcs which measure such angles do not differ very sensibly from their tangents, hence the ratio of two such angles may be said to be the same as their tangents.

[2] To those who require a further proof of these two assertions the following may be acceptable:

1. In Fig. 19 let AB be the object in one position and *ab* the other. For convenience these are shown in such positions that the line OC passes at right angles through their central points C and *c*. It is sufficient, however, that *ab* and AB should be the bases of isoceles triangles having a common vertex at O. AB is virtually an arc of a circle described from O with radius OC. Likewise *ab* is an arc of a circle whose centre is O with radius O*c*. Therefore—

$$\text{AOB} : a\text{O}b = \frac{\text{AB}}{\text{OC}} : \frac{ab}{\text{O}c} = \frac{1}{\text{OC}} : \frac{1}{\text{O}c};$$

hence AOB varies inversely as OC.

2. Two objects are placed at the same perpendicular distance OC from

Let x represent this distance, f the focal length of the lens C—Fig. 21 being considered—and d the distance of distinct vision of the observer figured as that between C and K. Then the pencils of rays proceeding from the object AB at the distance x must, after passing the lens C to form the virtual image, have the same divergence as though they proceeded

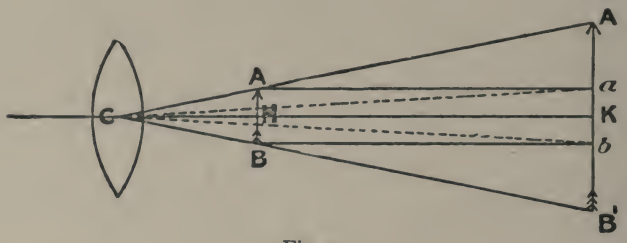


Fig. 21.

from A′B′ at the distance x. The condition may be expressed, then, by the following formula:

$$x = \frac{df}{d+f} \left(\text{derived from the well-known optical formula } \frac{1}{d} = \frac{1}{x} - \frac{1}{f}\right).$$

If the object AB were viewed by the *unaided* eye—that is, without the lens C—it would have to be placed at *ab*, because that is the plane of best vision for the observer, and so would be seen under the angle *aCb*; but with the lens *in situ*, it is

the eye O of the observer as in Fig. 22, AB and A′B′ respectively. They

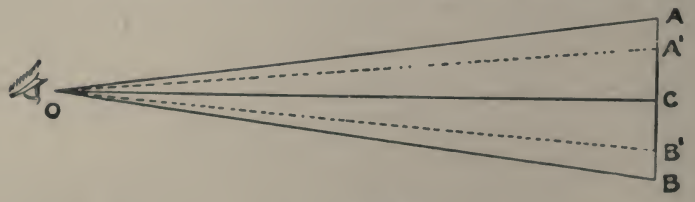


Fig. 22.

may be called arcs of a circle whose centre is at O with radius OC; therefore—

$$\text{AOB} : \text{A'OB} = \frac{\text{AB}}{\text{OC}} : \frac{\text{AB}}{\text{OC}} = \text{AB} : \text{A'B'},$$

which explains the situation sufficiently.

seen under the angle ACB, being mentally referred to the distance of distinct vision. It consequently appears of the same magnitude as though it really occupied the space A′B′, the virtual image. The ratio A′B′ to AB, then, is the measure of magnification as before explained, and that is the same as A′B′ to *ab*, or CK to CH, and finally as d is to x expressed as $\frac{d}{x}$.

It has just been shown, however, that—

$$x = \frac{df}{d+f}, \text{ hence } \frac{d}{x} = \frac{d+f}{f},$$

which expresses the magnification of an object for the distance of distinct vision d.

It follows, then, with normal vision the magnifying power may be properly represented by the fraction $\frac{10+f}{f}$, bearing in mind if a long-sighted person of 20 in. distance of distinct vision, that the 20 has to be placed in the numerator instead of 10; and if a short-sighted one of 5 in. distance of distinct vision, then that 5 be substituted for that figure.[1]

Before quitting the subject of magnification, the following remarks may be read with interest to the microscopist: As the actual magnification of an object is directly proportional to the angle subtended by it to the pupil of the eye, so it is said to be in the inverse proportion to the distance of such object *from* the eye. The observer, then, that is short-sighted, say at 5 in., may be said to have a vision four times as *strong* (for want of a better word) as one whose distance of distinct vision is 20 in.; for with an extremely small object, which the short-sighted individual might be just able to see with his naked eye at 5 in, the long-sighted cannot see at all unless he uses a lens of $6\frac{2}{3}$ in.

[1] For lenses of *short* focal length the expression is often written as $\frac{10}{f}$, which is practically correct; but owing to this statement having gained admission into several books as true for all lenses, it has been inferred as correct for lenses *even of long focal length*, which evidently is an error; for if such were true, say with a lens of 10 in. focal length, the result would be no magnification at all, but worse still if the focal length were longer, for then it would seem as if a *diminution* rather than an enlargement took place. The simple magnification formula, however, is rigorously correct for positive lenses of any focal length *if the eye be placed at the posterior focal plane.* See Appendix.

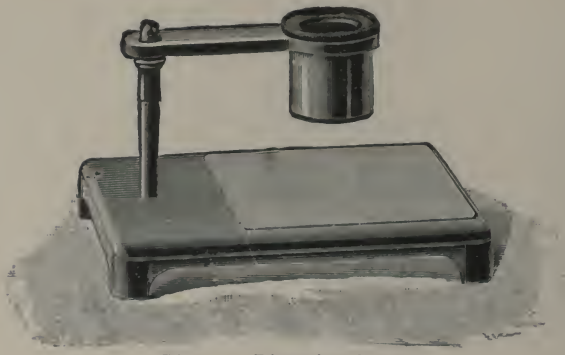


Fig. 23.—Dissecting Stage.

focal length, which means he has to use a lens to magnify four times before he can examine it as well as the short-sighted observer at 5 in. does with his naked eye. As an illustration, supposing we

Fig. 24.—Large Dissecting Microscope.

have a lens of half an inch focal length, taking the formula $\frac{df}{d+f}$ we find the place of the object, to be viewed distinctly, should be $\frac{5}{11}$ in. from the lens for the short-sighted observer, with $d = 5$, and $\frac{20}{41}$ in. for the long-sighted individual at 20. Consequently with the lens in question the object then would have approximately the same angular measurement with both individuals. By this we see that with lenses of *high powers* all

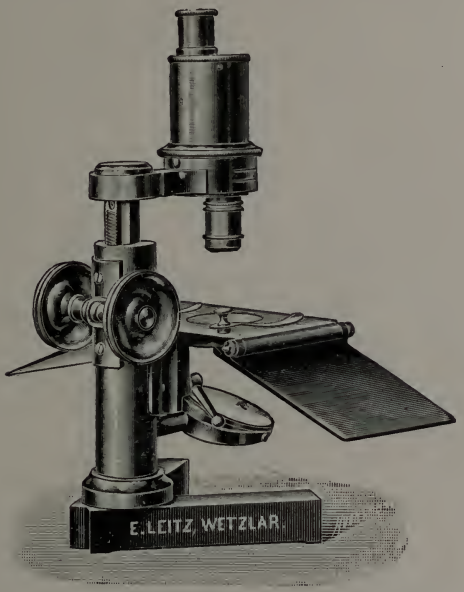


Fig. 25.—Dissecting Microscope with Erected Image.

observers, whether short, normal, or long-sighted, see objects with nearly, if not quite the same facility and distinctness.

The simple microscope is used in two forms, the one known as a dissecting microscope, of which there is an endless variety by different makers, and the other as a hand microscope.

The dissecting microscope is used more especially for the purpose of preparing specimens for the microscope; and as both hands are needed for this purpose, the actual optical portion of the instrument is held by a suitable support. Figs. 23 and 24 show different varieties of arrangement. The next two illustrations, Figs. 25 and 26, are added among this type of microscope

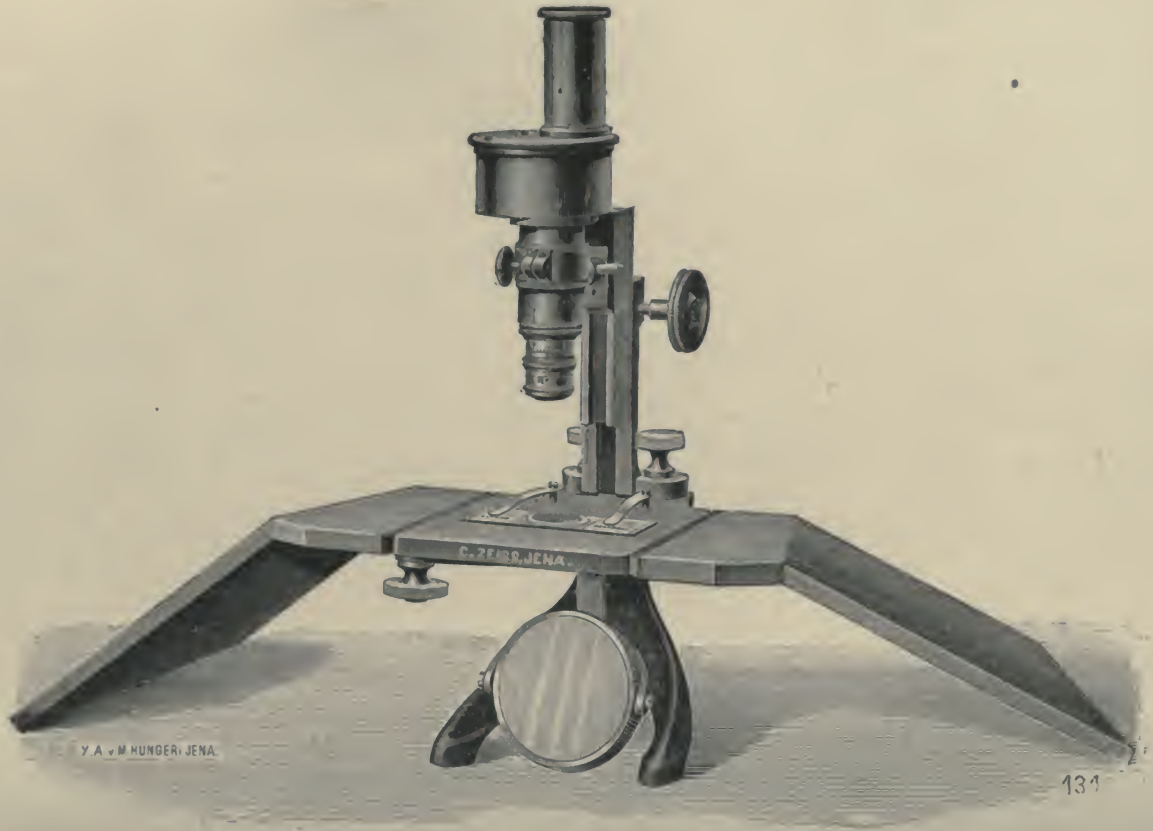


Fig. 26.—Particularly solid form of Dissecting Microscope with Arm-rests on the Table.

because they are used for the same purpose as the preceding, but optically speaking they are in reality *compound* instruments with erecting eyepieces.

The **hand microscopes** are intended, as their name implies, to be used by the hand only, and are convenient for use by medical men in examining skin diseases, and for other purposes which readily suggest themselves to the reader. The highest class of microscopes of this description are the "loups" of Zeiss, fixed in a handle, as shown in Fig. 27.

Fig. 27.

They are made of different magnifying powers from 6 to 30 times, all dropping into the same handle.

Mr. Nelson has designed a special form of hand magnifier fitted with an extended lens silvered on the back, which causes the magnifier to reflect light upon the object under examination and so to illuminate it. At times this may be a most useful arrangement. It is illustrated in Fig 28.

Another very handy form of variable magnifier has been brought out by Messrs. Watson & Sons very much after the fashion of the "Brucke" type, much used on the Continent. It magnifies from 5 to 10 diameters. The working-distance is quite exceptional, being, it is said, 2 in. with the minimum magnification. Its size, 3 in. × 1⅜ in., renders it inconvenient for pocket use, but it is a most useful adjunct to the consulting room or the laboratory.

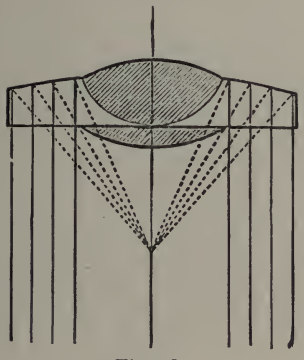


Fig. 28.

Lastly, Koristka of Milan has designed a *compound* hand magnifier giving a very great range of amplification (30 to 90), a large frontal distance, and an extensive field of view.

CHAPTER III

THE COMPOUND MICROSCOPE

THE compound microscope is far more intricate than the simple, both in construction and use, and indeed is the microscope *meant* when the term is used in ordinary speaking.

There are two models, which in their primitive forms are very dissimilar in appearance, although, of course, they are both designed for a common purpose; but in recent years so many compromises have been made by different opticians embracing the usefulness of both types and avoiding the inconveniences peculiar to each, that many are now sold which save for the length of the tubes—to be hereafter explained—it would be difficult to say belong distinctly to either model. The long-tube class are usually called the English models, a classical pattern being that shown in Fig. 29, by Messrs. Powell & Lealand, whilst the typical Continental short-tube pattern may be represented by that made by one of the oldest firms of manufacturers abroad—viz. Carl Zeiss, illustrated in Fig. 30. In each case, for the assistance of beginners, the names are affixed to the different parts of the instruments.

It is convenient for the sake of description that a microscope be divided into two portions: the Mechanical and the Optical.

The Mechanical Portion.—This division includes the entire metal portion of the instrument, which may be said to consist of the following parts:

1. The foot and upright support, with joint for inclination.
2. The tube carrying one or more draw-tubes, with its nosepiece.
3. The body and the coarse adjustment.
4. The stage—simple, mechanical, and auxiliary.

By Powell & Lealand.

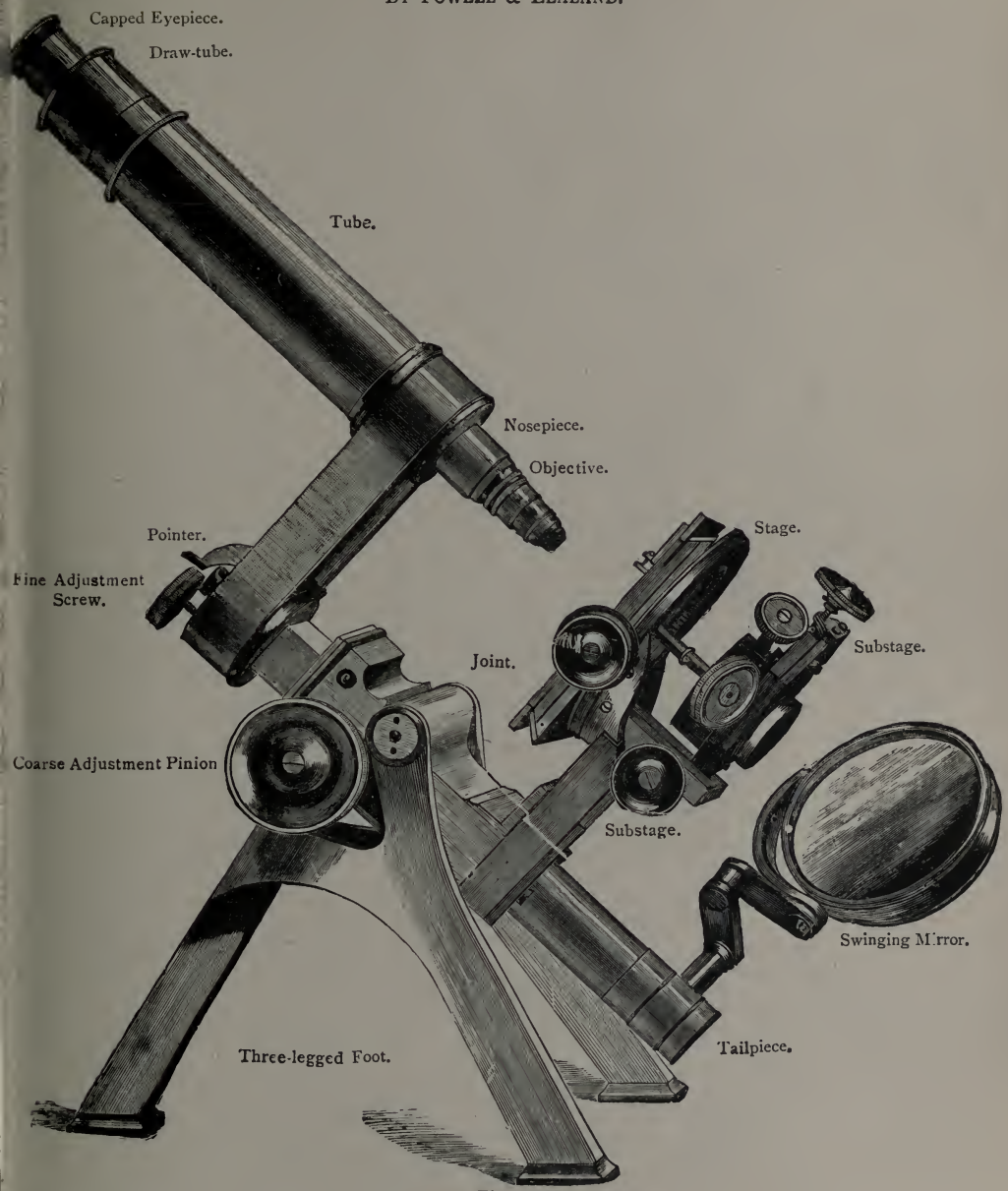


Fig. 29.

By Carl Zeiss.

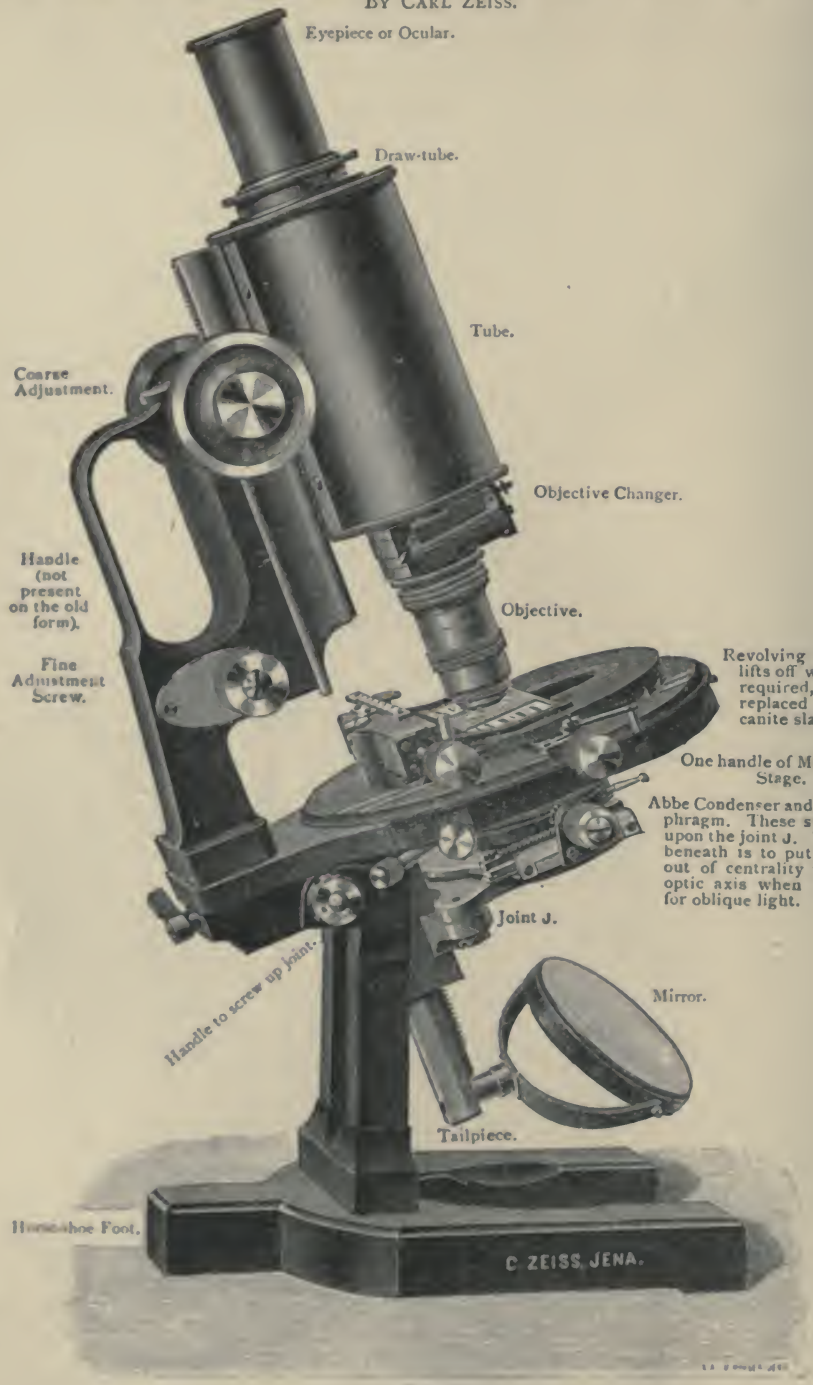

Eyepiece or Ocular.
Draw-tube.
Tube.
Coarse Adjustment.
Objective Changer.
Handle (not present on the old form).
Objective.
Fine Adjustment Screw.
Revolving Stage: lifts off when not required, to be replaced by vulcanite slab.
One handle of Mechanical Stage.
Abbe Condenser and Iris Diaphragm. These swing out upon the joint J. The rack beneath is to put the iris out of centrality with the optic axis when required for oblique light.
Joint J.
Handle to screw up joint.
Mirror.
Tailpiece.
Horse-shoe Foot.
C ZEISS, JENA.

Fig. 30.

5. The substage, including the diaphragm and mirror.
6. The fine adjustment.

1. *The Foot* may be three-legged, as shown in Fig. 29, or the horse-shoe shape, as in Fig. 30. Other forms are made, but are not now so much in favour as the two mentioned. In the English pattern the union of the two front legs, it will be seen, is so arranged that a joint is made between them at which the instrument can be inclined or placed completely horizontal; whilst, in the Continental model, two supports usually rise up vertically from the horse-shoe which end in a pivot arranged for the same purpose. A handle on the pivot, or some other simple arrangement, is usually provided to tighten the joint when through use it becomes loose.

2. *The Tube* is seen to be longer in the English form than in the Continental; but in each model a supplemental one—called the draw-tube—is contained within it, by which means the entire "draw"[1] can be lessened or increased. The extreme draw of the English, or long-tubed instrument, is usually about 12 in., whilst that of the short tube, or Continental model, is limited to about 8 in. It is rapidly becoming the fashion, by the use of a third tube, usually articulating by means of a special rack and pinion with the *draw*-tube, to make the instrument capable of being extended to 12 or 13 in., but closed to 5 in., a very great convenience, as will hereafter be seen.

The end of the tube that does not receive the draw-tube is provided with a fitting called the nosepiece, which is a casting turned out to ·8 in. diameter, and threaded with a screw thirty-six threads to the inch. This combination of diameter and thread is called the "universal fitting," and all objectives, both English and Continental, are now made to fit the same. A similar nosepiece, but of a very much lighter kind, is attached to the end of a draw-tube that is contained within the tube. Its use will be described later on.

The Draw-tube,[2] usually divided into millimetre divisions, should slide in its fitting within the tube easily and smoothly, but the cloth fitting, which Messrs. Bausch & Lomb provide, is

[1] The "draw" of a microscope is usually measured from the upper end of the draw-tube to the end of the nosepiece.

[2] Internal diameter, small size, ·917 in., or 23·3 millimetres. Large size, 1·27 in., or 32·25 millimetres.

a very great addition, for it seems it does not get dry and stiff like the plain spring-brass one usually supplied.[1]

The diameter of the tube and draw-tube is greater in the English pattern (and, of course, the oculars too) than in the Continental make; but the American manufacturers, Messrs. Bausch & Lomb, seem to have adopted an intermediate size, which may have its advantages.[2]

The Royal Microscopical Society has laid down certain measurements for different-sized draw-tubes, which in future years will probably be of much service. At present the arrangement does not facilitate the interchange of oculars; hence purchasers of eyepieces and other fittings that are dropped into the draw-tube should be careful in stating its exact diameter when sending orders by post, Messrs. Zeiss recommending an impression in wax being taken to prevent mistakes.

3. *The Body* articulates with the tube of the instrument by means of the *coarse adjustment*, which consists of a rack and slide fixed to the tube that fit into a corresponding slide containing a pinion, forming part of the body. Several different forms of construction are adopted by different opticians in the details of this arrangement, but all are built and manufactured to obtain a smooth movement from one end of the rack to the other, and an absence of what is called "*back-lash*," a name given to a loss of way that used to be present in the old forms of manufacture, when the raising of the tube was changed to a lowering movement. This is largely brought about by the use of a diagonally cut rack and spiral pinion. At both ends of the pinion are two large milled heads seen in both Figs. 29 and 30. Coarse adjustment is made by turning these milled heads. It should be remarked the exact and accurate movement of the coarse adjustment constitutes one of the points to be carefully looked into in purchasing a microscope.

In the common forms of the instrument the coarse adjustment is done away with, the tube drawing through a sleeve which is attached to the body instead. This type of microscope does not lend itself to the use of high powers. Whatever the type

[1] How to ease this fitting when becoming stiff, is found in the chapter upon "Hints for Faults" at the end of this book.

[2] A full discussion of the relative merits of the Long- and Short-tube instrument, taken as a whole, is furnished later on.

of instrument possessing a coarse rack motion, such should be of the best description and accurately fitted. It is well, if any doubt exists upon the point, to rack the tube completely out and see if the slides (and rack) are filled with a heavy grease—if this be so it is a bad sign, for it is probably there to cover up a bad and loose fitting.

4. *The Stage* may be simple or compound (sometimes called mechanical). The former merely consists of a solid piece of thick brass fixed at right angles to the body, in such a position beneath the end of the tube that takes the objective, that it affords a convenient support to the specimen. It is perforated in the middle by a large hole for the passage of the light from the mirror. Two little springs are supplied with this form of stage for clipping and holding the specimens firmly to it. Some manufacturers make a broad slit completely through the stage on the side furthest from the body of the instrument, converting the circular hole mentioned into a U-shaped opening. It serves the purpose of permitting the microscopist, when focussing with a high power, to lay hold of the slip containing the specimen, between his thumb and finger, so that by lifting it up and down *very* gently from the stage he can mentally estimate the distance it lies from the front of the objective in use. Underneath this simple form of stage may often be seen a sleeve which is to hold a substage condenser or a diaphragm.

In the best form of instruments, however, the stage is more complicated and is arranged so that, by turning two screws furnished with milled heads, two motions shall be available. These movements, to an observer looking through the instrument, are up and down at right angles to the optical axis and from side to side. In the English model this compound stage, with its mechanical superstructure of slides and screws, is usually square-shaped, but in the Continental it is more often round. In either form, with instruments of the highest grade, the movements to and fro and from side to side are all capable of being noted by verniers with graduations reading to either portions of a millimetre or of fractions of an inch. These afford a means of registering some particular part of a specimen[1] for future reference. Besides these two motions, however, in the very best

[1] How to do this is explained later in a chapter devoted to the purpose and to the discussion of the units of measurement used by microscopists.

of instruments it is usual nowadays for the entire superstructure to revolve on the optical axis of the instrument, so that a specimen may be turned round in any direction desired, a vernier being again added to register the amount of circular movement.

To make a stage turn about the optical axis *truly* is no small matter, and in some imperfectly made instruments the act of turning causes the specimen to quit entirely the field of view; it is usual, therefore, in the finest constructed apparatus, to add centring screws to adjust the stage to optical centrality when necessary. In the English stands we regret to say this adjustment is often conspicuous by its absence, whilst in the best Continental it is nearly always present. Very little reflection will enable the reader to understand that if the stage becomes *ec*centric through use, it upsets the values previously obtained by the verniers, and notes on different slides to indicate places of special interest become valueless in consequence; hence the microscopist has to hunt again over the specimen to find the desired position, which practically does away with one of the objects of the mechanical stage. If, however, the stage can be always kept central—especially before noting a new position—or can be immediately reset to absolute centrality by means of the screws in question, the present readings taken, as well as all the old ones on other slides, will always be correct and correspondingly useful.[1]

Whether the stage is better in its round form or as a square or of a rectangular shape is a matter of opinion. The round form facilitates the recording of circular motion about the optical axis, although in the best English stands, even with their square stages, some means is usually provided which answers the purpose.

The size of the stage varies somewhat according to what special use the microscope is to be applied; for instance, in bacteriology it should be exceedingly large, but this matter is discussed later on when considering the class of microscope best suited for special purposes.

In many instruments arrangements are provided to take up the wear and tear in the different slides of the mechanical stage and also the loss of way in the screws which produce the different movements. If not abused, these are very useful,

[1] How to set the stage "central" is fully explained later on.

By Charles Baker.

Both rectangular movements are effected by rack and pinion, the vertical one of which carries a bar (fixed as to horizontal movement) against which the slide is pressed by a spring clip, and upon which is mounted the rack and pinion for the horizontal movement; the points which press upon the slip are tipped with cork in order to grip the slide, and move it along the fixed bar, when the milled head is rotated; the slide actually rests on two small raised surfaces at either end of the bar to minimise friction.

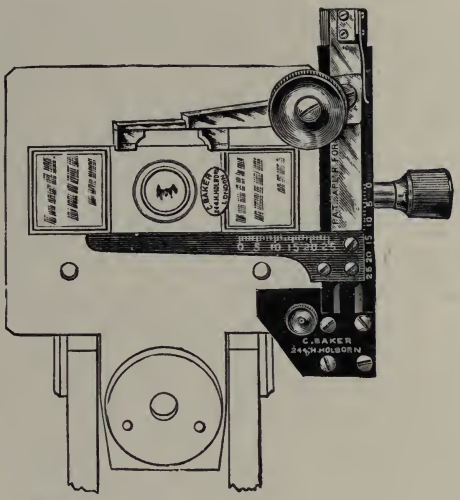
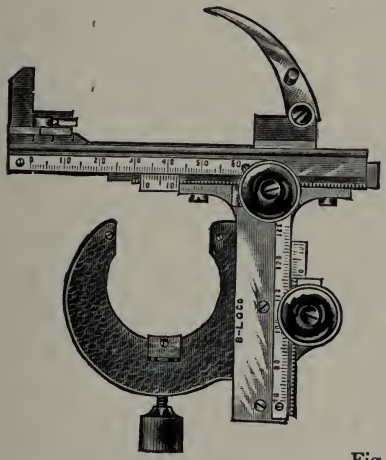


Fig. 31.

but many manufacturers have found adjustments of this nature to have been so unfairly or ignorantly employed, that injury

By Bausch & Lomb.



The rectangular movements are both by rack and pinion, as it is impossible to make a worm-screw movement that will not wear loose in time. The object slide rests upon the surface of the microscope stage, and may be used in immersion contact with the condenser if desired.

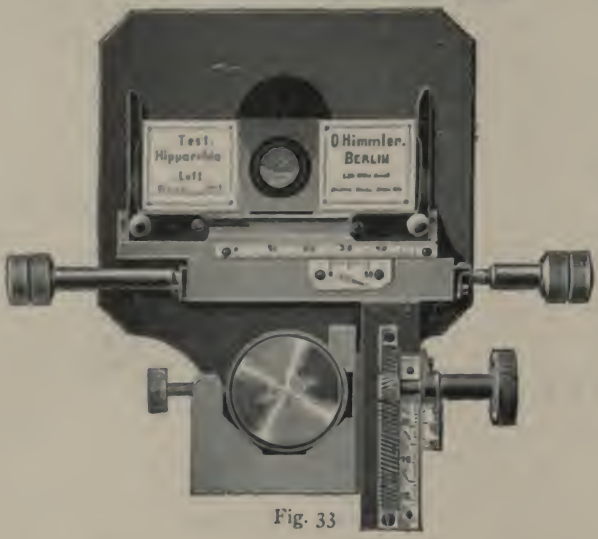
The stop against which the slide rests is adjustable, permitting the use of slides of various sizes. The object carrier has extra long range, the movements being 35 and 60 mm. respectively.

The stage is held in place on the microscope by a solid metal clamp.

Fig. 32.

to the slides of almost a permanent nature has resulted; consequently they now no longer add them to their instruments.

By Otto Himmler, Berlin.

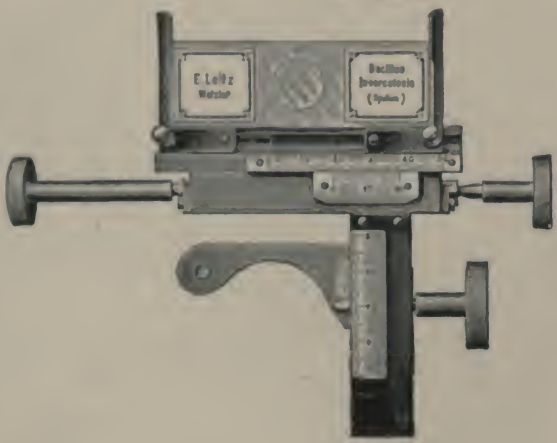


In this arrangement one movement is by an endless screw, whilst the other is by rack and pinion. It can be clamped to the microscope quite easily.

Fig. 33

All stages hitherto considered have been built into the stand, in other words are fixtures forming part of the instru-

By Leitz.



This arrangement and the preceding are very closely allied; the different *shape* of the milled heads is preferred by some microscopists.

Fig. 34.

ment itself; but Carl Zeiss and many Continental firms however, in their circular stage, make the top plate to lift off to

be replaced by a vulcanite one when using corrosive fluids. This has been found of great service, for, if such should run on to the metal, they injure it very sensibly.

With microscopes fitted with plain stages, the loss of a mechanical one is very much felt at times—such as when making a blood-count, for example, or hunting very systematically through a specimen; hence opticians meet this want

By Swift & Son.

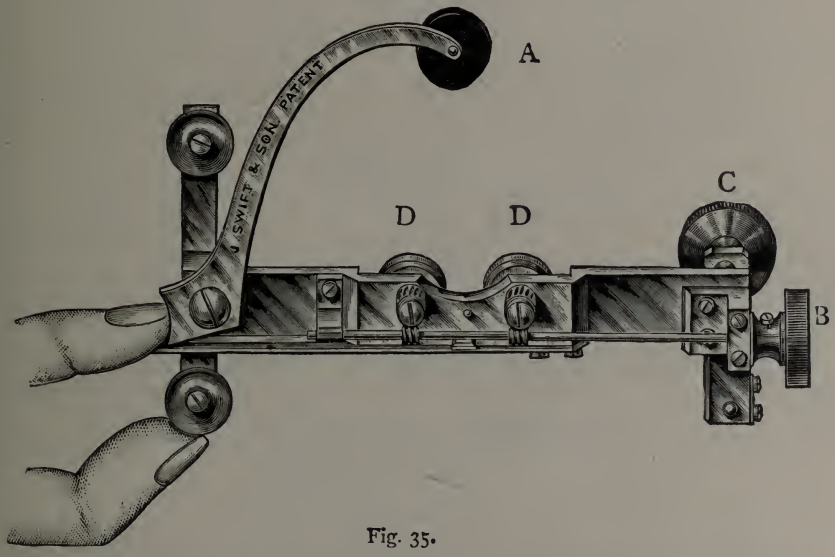


Fig. 35.

This engraving shows the peculiarity of the construction and the best method of raising the lever, carrying the roller A, which, when released, presses the slide under examination against the friction rollers D, D. The milled head B works the friction rollers D, D, and gives 2 in. traversing motion to the slide under examination. The milled head C allows of a vertical movement to the same extent.

by supplying an attachable device which used to be called after the inventor Mr. Mayall, junior, although he is now rarely credited with the originality. We do not recommend any of these arrangements in preference to the previously mentioned substantial form for regular use, but as a go-between they are decidedly useful. Illustrations are given (Figs. 31 to 37).

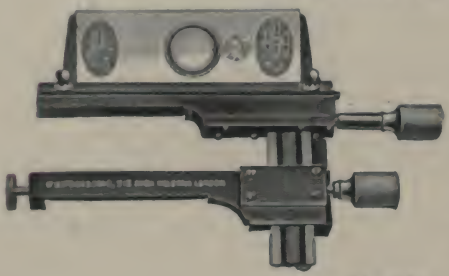
5. *The Substage.*—The substage[1] assumes a variety of forms according to the individuality of the manufacturer. Primarily

[1] Standard size of internal diameter of fitting is 1·527 in. = 38·786 mm.

we may divide all kinds into two grand classes—the English and the Continental.

In the cheap make of either type the substage *proper* is really not in evidence at all, its only representative being a sleeve (made of the correct diameter to receive an Abbe or other condenser with or without an iris diaphragm) and attached to the undersurface of the stage instead. "Up and down" motion, as it is called, by which is meant bringing up the condenser nearer to the slip containing the specimen placed on the stage of the microscope, or carrying it away further from it, is performed by pushing the condenser further into the sleeve, or by pulling it out again by hand, unassisted

By Watson & Sons.



The special feature of this stage is that it can be immediately fixed to a microscope without any special fitting. It is placed upon the stage and grips upon the edges like an ordinary sliding bar; it is then clamped in position by means of a thumbscrew. It has a long range of movement in both horizontal and vertical directions.

Fig. 36.

by any mechanism whatever. The whole arrangement is crude, but is sufficient to be in accordance with the general scope and design of what may be called a somewhat primitive form of instrument.[1]

In a rather better class of microscope, however, the sleeve of which we have just spoken is not itself attached to the stage beneath, but is contained in a fitting of its own of simple design, furnished with a kind of thumbscrew by means of which it is held to the stage. The screw when turned between the thumb and finger raises or lowers the fitting containing the condenser, but its construction and that

[1] The reader who is commencing the subject, must not confuse the "up and down" motions of the *stage* with those of the substage. The former by convention always means in a direction at right angles to the optical axis, the latter parallel with it.

of the fitting is such that when the condenser is not required to be in use the whole fitting can be rotated upon this screw and turned aside out of the way. The return of the fitting to its original position is notified by a click, by which the

By Carl Zeiss.

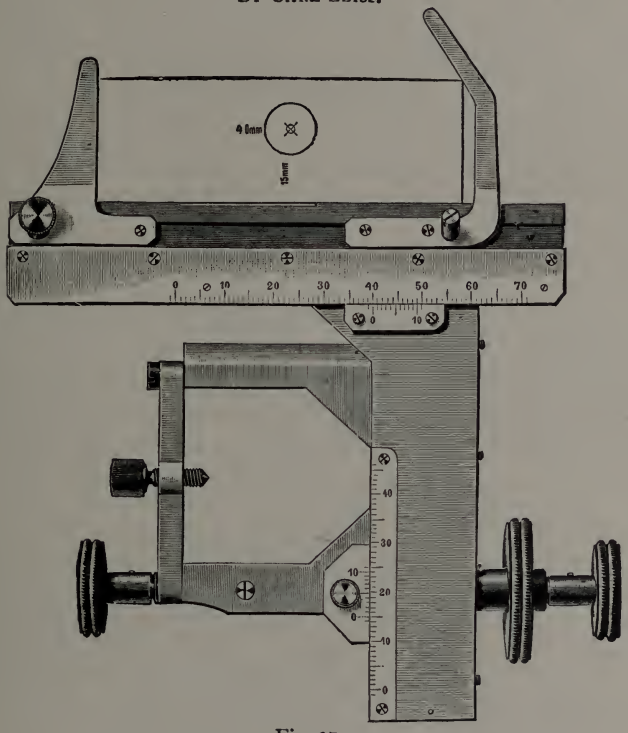


Fig. 37.

The milled heads which cause the movement of the sliding pieces are co-axial in this pattern of traversing stage and retain their relative positions in turning. The lateral movement may be effected with either the right or left hand.

The peculiar mechanism of this stage guarantees a considerably greater security in the movement of the sliding pieces and fulfils all requirements of a movable stage when applied to the purposes of a "finder."

microscopist understands the condenser is once more replaced in the optical axis of the instrument. Owing, however, to the fitting not being usually provided with the means of accurately centring the condenser, such as we shall shortly explain, this

"return to centrality" with the optical axis must only be regarded as an approximate effort, as the whole arrangement is somewhat rough. Hard wear is apt to spoil the efficiency of this design, so that when the microscope is intended to be

By Watson.

Fig. 38.

employed in a laboratory where frequent and sometimes rather rough use is to be expected, we do not recommend its adoption. We have known it more than once become very troublesome and shaky, so that when the fitting was "clicked" home the

By Watson.

Fig. 39.

By Watson.

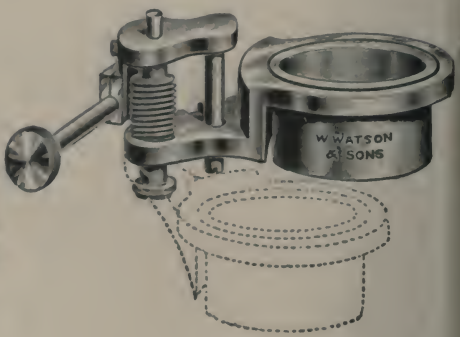


Fig. 40.

condenser was anything but central, a condition of things quite sufficient to lessen seriously the good performance of the objective. The arrangement may be seen in Fig. 38, other varieties being shown in Figs. 39 and 40.

To be able to centre the condenser in these cheap stands, then, is a desideratum, and Messrs. Swift & Son, recognising how important such a process is, have introduced a neat little fitting of great excellence and just suitable for the special object in view. It is shown in Figs. 41 and 42 differently mounted.

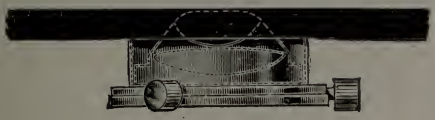


Fig. 41.

A handy contrivance by SWIFT, arranged to fit into the ordinary tube fitting beneath the stage in cheap microscopes.

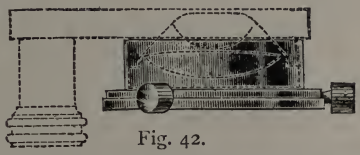


Fig. 42.

Arranged to be slipped into the sleeve of the cheap understage fitting of a better type. Also by SWIFT.

In the *best* form of English instruments, however, the mechanism of the substage is far more complicated. The body of the instrument is seen (Fig. 43) to be prolonged into what is technically called a "tailpiece," which is provided with

BY WATSON.

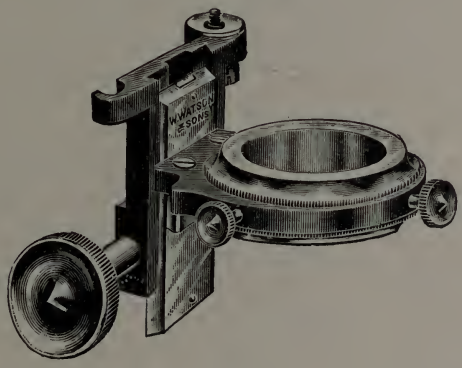


Fig. 43.

The Substage as fitted to the Edinburgh Student's Microscope.

a carefully cut rack, the engaging pinion being fixed in the illuminating apparatus itself, which thus enables the operator to raise or lower the whole substage with ease, delicacy, and precision. In some instances an auxiliary arrangement called

a fine adjustment to the substage is also added to enable the microscopist to perform these up and down motions with still greater delicacy. Later, when speaking of the use of the instrument taken as a whole, we shall point out a change in our own opinion of recent years; for we used to recommend this extra fine adjustment, whereas we now believe it a quite unnecessary expense. Three small, milled headed screws (two of which are shown in Fig. 43) are usually to be found in the ring into which the optical portion of the condenser drops; they serve for the purpose of accurately centring the condenser with the optical axis of the instrument. Special diaphragms of particular design which used to be in vogue, or coloured pieces of glass can be dropped into the additional ring placed beneath the condenser (not shown in the cut) which is made to turn in and out especially for the purpose. The iris diaphragm is placed beneath this ring. It is provided with a little handle to open and close the leaves, whilst graduations to show how much they are opened or shut may often be found in a suitable position.[1]

In the English model this iris diaphragm is usually rigidly fixed into the illuminating apparatus, by which we mean it *cannot* be shifted when so desired *out of the optical axis*, special care being exercised by the maker, so that when a microscope is sent out the iris, when reduced to a pinpoint, shall indicate the actual optical axis of the instrument. But we shall show later on that this is exactly opposite in the case of the Continental form of illuminating apparatus, which is made entirely different in this respect.

The mirror (concave and plane surfaces) in the English type of substage is usually held to the tailpiece fixed in the body of the instrument, by a double-jointed arm permitting movement in all directions.

The Continental form of substage is very different in design. The most usually adopted type is that originally devised by the late Professor Abbe, being first made by Carl Zeiss; but it is copied by nearly all Continental manufacturers (Fig. 45). It has advanced in favour so much in recent years with workers in this country, that several English opticians in addition are adopting the design (notably Messrs. Watson & Sons) with new

[1] See article upon the use and abuse of Substage Condenser.

models of short-tube microscopes that they have brought out ; at least they have constructed these so that they are immediately capable of receiving this class of substage when desired. After several years of close acquaintance, we are bound in common justice to say we are more than satisfied with this arrangement. For excellence of workmanship and neatness of design, we are certain it has no superior ; but whilst saying this we are bound to admit there is one omission in its construction we have always regretted, and that is the absence of centring screws to the sleeve of the condenser. It is true the Abbe *achromatic* condensers, as made by the same firm, have each their *own* self-contained centring arrangements to make up for this deficiency

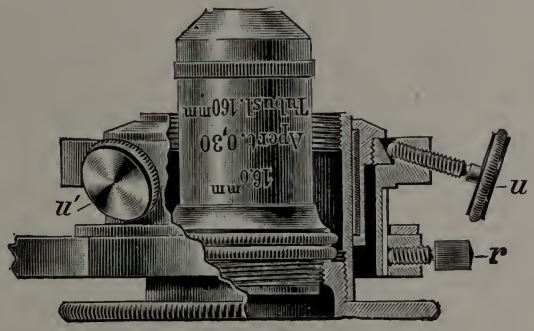


Fig. 44.

Centring appliance for Objective as Condenser (full size).

of which we complain, but surely it is an unnecessary expense to have this in addition to pay for, with *each condenser*, when one on the substage would do for all. To remedy this omission the firm sell a centring fitting which drops into the sleeve of the condenser, which, though primarily designed for using objectives as condensers (and so in consequence supplied with the "universal fitting," the same as the nosepiece), will take an English condenser just as well as an objective (Fig. 44). Messrs. R. & J. Beck make a similar class of fitting which we have used for years with great pleasure ; we want nothing better. Neither of these self-centring devices can be used with the *chromatic* Abbe condensers sold by Zeiss, because they are of too great a diameter. The consequence is they cannot be centred with the Continental microscope, which seems a serious

oversight and one to which we very earnestly call the attention of the Continental opticians.[1]

The whole illuminating apparatus is raised or lowered by turning a neatly milled handle (S in Fig. 45), which actuates a pinion that engages in a rack let into the tailpiece, easily seen in the figure.

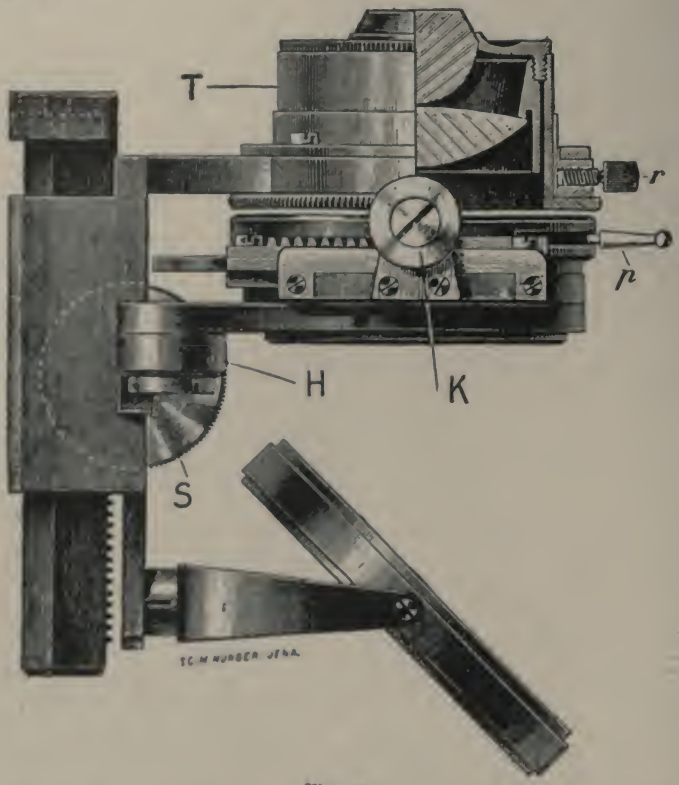


Fig. 45.

The whole substage may collectively be said to consist of three parts: (i) a sleeve to hold the condenser,[2] (ii) an iris diaphragm, and (iii) a mirror.

[1] We admit that most Continental chromatic condensers can be purchased mounted in simple tubes, but the great size of the illuminators forbids their use with arrangements we have mentioned.

[2] It is useful to know the exact diameter of this sleeve is, in the Zeiss microscope, 36·8 mm.; Reichert and the R.M.S. are similar, viz. 38·786 mm. or 1·527 in.; whilst Leitz is less than either, being 30·5 mm.

(i) Little need be said of the sleeve T save that it contains no centring adjustments.

(ii) The iris diaphragm, opened and shut by the handle *p*, is contained in a fitting of its own which swings in and out by the strongly made arm pivoted to the substage at H. This is done by *pulling upon* K *in an outward direction*, and is required when using polarised light for the polariser to be dropped into position, or for the addition of stops or coloured glass to be used as filters. On returning the iris into its former position, a distinct click is heard when it is central. The handle also serves two other purposes. If *turned* between the thumb and finger it places the iris diaphragm *eccentric to the optic axis*, which it does by causing the frame containing the diaphragm to run along the rack seen (apparently) by the side of the handle K. In addition, if pushed *around* the optical axis to make the handle move towards *p* in the diagram, it causes the iris diaphragm to revolve in the same manner; hence if the iris be set *eccentric* to the axis, it will describe a circular movement about the field by the means of this handle.

(iii) The mirror, which has one face plane and the other concave, is fixed to a tailpiece projecting from the lower end of the illuminating apparatus. It can be lifted off when not required. In the very latest pattern of the Zeiss stand, the mirror enjoys a limited up and down motion on the tailpiece which it did not possess in the original type of the firm's manufacture.

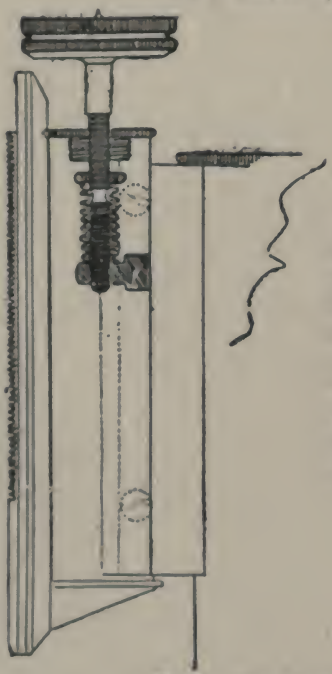
Such may be considered as the general outline of the Continental substage, but it is to be understood that individual makers add or alter certain details in accordance with their own liking.

Whether the mirror be for the Continental or English stand, it should be made of very thin glass, with parallel faces, so as to avoid, as far as possible, double reflections. The use of a glass right-angled prism for this purpose theoretically is good, seeing that all double images are done away with, but practically most microscopists find its use more trouble than good, as it is of necessity so bulky and can mostly only be used with the instrument when in an erect position.

6. *The Fine Adjustment* is perhaps the most important of the mechanical details for a good microscope. There is some

difficulty in obtaining a consensus of opinion as to whether it should have a fairly rapid screw or one exceptionally fine. It would seem that for certain purposes where the magnifications are not what the microscopist calls very high, too slow a screw is troublesome; yet on the other hand where the size and (probably) the depth of objects are commensurate with the

By C. Baker, 244, High Holborn, London.



This consists of two threads of different pitch in the micrometer screw. The screw nearest the milled head has forty threads to the inch, and works through a fixed socket; the other screw of fifty threads to the inch works through a movable socket which is attached to the body of the microscope.

The effect of revolving the milled head is to raise or lower the first screw forty turns to the inch, whilst the other thread causes the socket carrying the body to travel at the rate of fifty turns to the inch, the speed of the fine adjustment being $\frac{1}{2000}$ of an inch for every rotation of the milled head.

Fig. 46.—The Campbell Differential Screw.

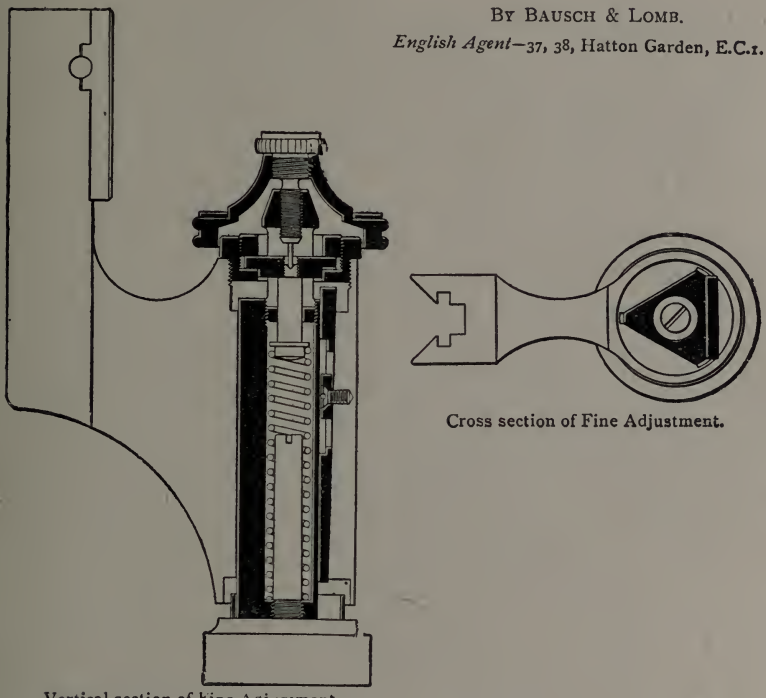
wave-length of light, it would be possible to have a fine adjustment with too slow a pitch of screw. In photomicrography the same difficulty is met with. Users of low-power objectives do not always like slow-pitch fine adjustments; it seems as if they never felt certain when the object was in focus on the ground-glass screen; yet on the other hand, when employing a magnification of at least a thousand or two thousand diameters, or even more, the finer the adjustment the better.

An arrangement by which both requirements are satisfied is

that by means of a double head (one small and the other large), *a quick or slow motion* of the screw is effected. It is supplied by several firms nowadays.

Whatever the arrangement adopted, one of the leading points

By Bausch & Lomb.
English Agent—37, 38, Hatton Garden, E.C.1.



Cross section of Fine Adjustment.

Vertical section of Fine Adjustment.

Fig. 47.

The micrometer screw acts directly upon the fixed triangular portion of the arm, the weight of the body of the microscope being balanced by a spiral spring, and the screw is thus subjected to a strain equal only to the friction or resistance in the adjustment, plus the difference between the tension of the spring and the weight of the body, as the spring is compressed when the adjustment is brought nearer to its limit of motion. Lateral motion is eliminated by an original device, which does away with set screws and springs, with their liability to relaxation and wear.

is smoothness of action. Another great feature is "stability when at rest." By this is meant that when an object is focussed, it should not go out of focus by itself (see hints at the end of this book), or even when the tube is tapped or the table rapped. To make the trial, Van Heurck's test-object is a good

By R. & J. Beck, Cornhill, London.

This is made upon a new patented method invented by Mr. Ashe. A strong lever C moves the cradle H, which carries the body of the microscope by means of the block D, which is a projecting portion of the cradle. The cradle slides in a fitting in the limb E, with a spring acting upon the upper side of the projection D, which drives it on to the lever C. The lever C is moved by a steel screw with milled head A; this screw works through an outer screw, which is provided with a large graduated milled head B, read by a folding indicator at F.

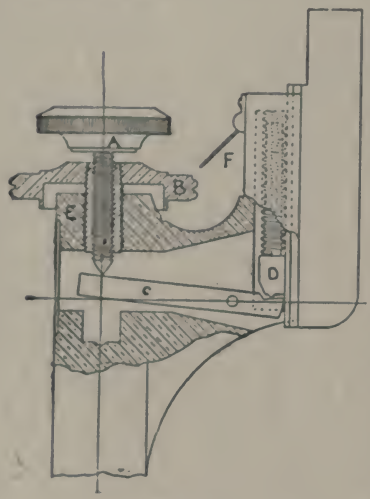


Fig. 48.

The screw A has a comparatively coarse thread, and when revolved raises the body a distance of $\frac{1}{20}$ of an inch in a complete revolution. The screw B has a fine thread, and when revolved carries the screw A with it, moving the body only $\frac{1}{200}$ of an inch in a complete revolution. At any moment either milled head may be used, giving a moderately fine adjustment for such powers as $\frac{1}{4}$ in. or $\frac{1}{8}$ in., or a very fine movement for $\frac{1}{12}$ in. or $\frac{1}{24}$ in. lenses. The convenience of such an arrangement can scarcely be overstated. By clamping the milled head A to the milled head B, or by clamping the milled head B to the limb E by screwing it tightly down, either one or the other fine adjustments may be put out of use.

By E. Leitz, Wetzlar. (*Ordinary Model.*).

This fine adjustment consists of a micrometer screw, which moves the tube and arm of the microscope, supporting the coarse adjustment vertically upon a triangular column. This column is virtually a continuation upwards of the upright support of the base of the microscope. Around the column is a broad collar so accurately fitted that it moves smoothly upon it with a minimum of friction and still without lateral motion, the movement being controlled by means of a micrometer screw at the top of the column. The head of this micrometer screw is milled, and is graduated in such a way as to indicate the exact degree of motion of the microscope tube, which is accomplished by turning the micrometer screw, each division of the graduation corresponding to a motion of the tube through $\frac{1}{100}$ mm., and a complete revolution of the screw corresponding to a motion of the tube through $\frac{1}{2}$ mm.

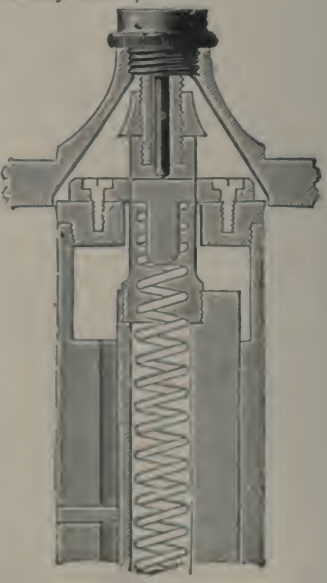


Fig. 49.

By E. Leitz, Wetzlar (*Special Form.*)

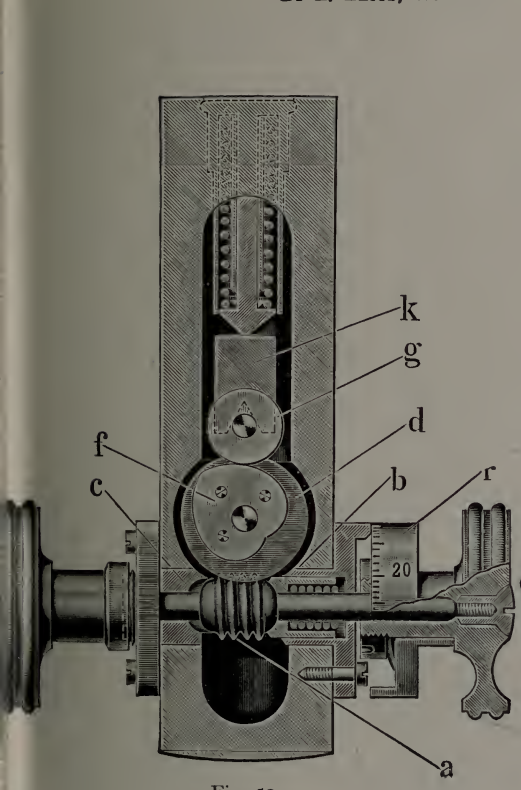


Fig. 50.

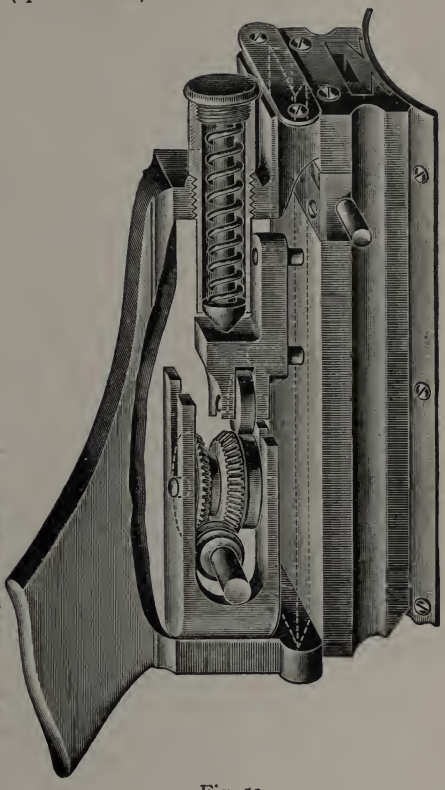


Fig. 51.

The two milled heads with drum are mounted on an axle *a* (Fig. 50), which in a central portion is enlarged and provided with a worm gear. The latter in its turn drives the wheel gear *d*. A spiral spring pressing against one of the journals into which the axle is mounted prevents any lost motion in the worm gear. Mounted on the same shaft with the gear-wheel *d* is a heart-shaped cam *f*. On this cam rests the steel roller *g*, fitted on the support *k*, which in its turn carries the microscope tube. By its weight and a spiral spring the latter presses on the cam *f*, and effects a direct vertical movement.

The periphery of the heart-shaped cam *f* is exactly symmetrical and mathematically correct. The distance traversed by its curved sides from the lowest to the highest point, and *vice versâ*, is 3 mm. Sixty teeth are cut in the entire periphery of the gear-wheel *d*, the number corresponding to one side of the heart-shaped cam or 3 mm. elevation being 30, therefore 1 tooth means a movement of $\frac{3}{30}$ mm. or 0·1 mm. Owing to the drum being attached to the axle *a* and its head divided into 100 divisions, each of these equals ·001 mm.

This adjustment (see also Fig. 51) is exceedingly accurate and reliable, and besides, it claims the following advantages:

By virtue of its unique construction the movement is continuous; there is absolutely no limit to the motion of the micrometer screw, which may be turned forward or backward as the work requires.

The movement extends in an exact ratio to the revolution of the cam for a distance of 3 mm. As another advantage we may mention it is almost impossible to break the cover-glass of the specimen, for should the objective come in contact with the cover-glass and assuming the knob is turned still further, then the tube, which is of aluminium and very light, would simply rest on the specimen without breaking the cover-glass, as the latter will easily stand the small pressure of the tube and the fine spiral spring.

By Messrs. Powell & Lealand, Emsdale, Greenham Road, London, N.
(*Late* 170, *Euston Road, London, N.W.*)

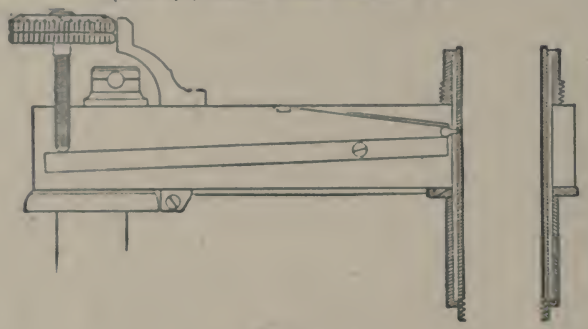


Fig. 52.

This adjustment, although devised some forty years ago, still seems to meet the necessities of the modern microscopist of the most exacting nature. The diagram explains itself, the construction being so exceedingly simple.

By Reichart, Wien.

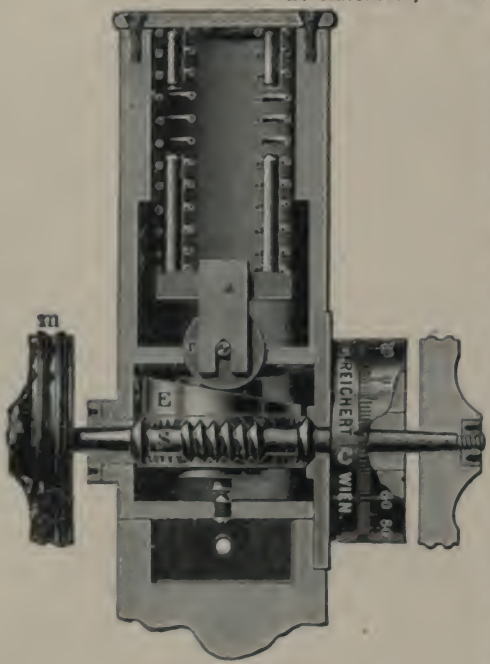


Fig. 53.

This fine adjustment is by means of a new micrometer screw which operates in the following manner:—By turning the milled head *m* a spindle on which a screw is cut actuates a worm wheel by the rotation of which a roller is raised or lowered, and with it the tube.

In this manner a fine adjustment of the greatest delicacy and durability is obtained. The movement of the micrometer screw is an endless one, which is a feature of considerable importance, as a backward movement is never required.

Since the only downward pressure is that of a delicate spring and the slight weight of the aluminium tube, the resistance to the micrometer screw is exceedingly small, and injury to the cover-glass is almost impossible, even should the objective come into contact with it.

All bearing surfaces are of steel, and the entire mechanism is protected within the frame of the microscope.

The head of the micrometer screw is so graduated that one division is equivalent to ·001 mm. movement of the objective.

By Swift & Son, Tottenham Court Road, London.
(*Old Model still used.*)

A, A (Fig. 54) the optical tube, which has soldered to it a gun-metal bar, sliding in "vee" grooves; B is a box at the back of the body, in which is fitted below a horizontal micrometer screw with a milled head F acting on a vertical lever of the first order D. C is a stud with a free running hard steel pulley fixed to the inner surface of the box B, which engages the top of the lever near the fulcrum. The sliding bar is provided with adjustments for taking up wear, so that side-shake and loss of time are impossible in this form of slow motion.

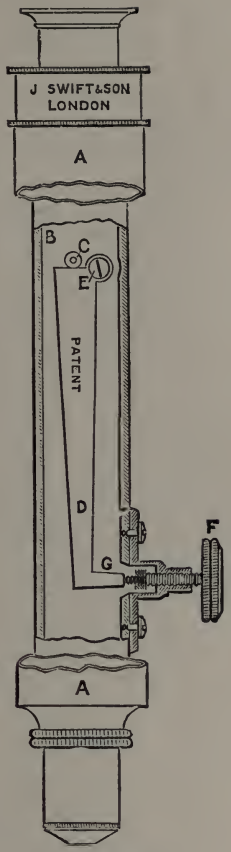


Fig. 54.

Swift's New Model.

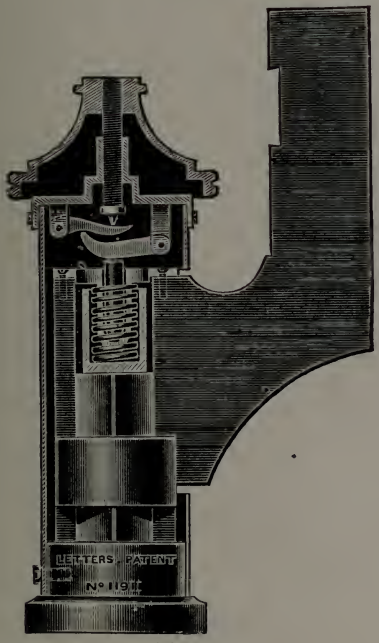


Fig. 55.

Fig. 55 (The New Model).—By an attachment to the head of the pillar, a delicate screw by means of a fine point of hardened steel acts by a series of levers to raise or depress the tube in a manner that affords the most refined movements devoid of all lateral shake.

By Watson & Sons, 244, High Holborn, London.
(*Standard Pattern.*)

The entire body is raised or lowered by means of a milled head fixed to a screw having a hardened steel point, acting on a lever (the contact surfaces of which are hardened and polished) against a point attached to the body-slide, in a perfect dovetailed fitting, about 2½ in. long.

At first sight it would appear that the screw controlling this important movement had to bear the entire weight of the body of the instrument. This, however, is not the case. The turning of the milled-head screw actuates a hardened steel lever B, varying in length according to the size of the instrument, the fulcrum C of which is placed as closely as possible to the sliding fitting in which the movement of the body takes place, whereby the weight carried by the milled head is considerably reduced.

Fig. 56.
Section of limb of Edinburgh Student's Microscope, showing construction of Watson's Standard Fine Adjustment.

Also by Watson & Sons.
(*Second variety*, Fig. 57, *with fast and slow motion.*)

The ordinary lever is shown with its fulcrum at D, its point of contact with the microscope fittings at C, and with the controlling milled head A touching the lever at E. By the use of a coarse thread to this milled head a speed is imparted of about $_{1}/_{10}$ in. = 250 μ for each complete revolution of the milled head A. An additional lever arm is attached to the ordinary one at F, and is actuated by the milled head B touching the lever at G. By the use of this the travel imparted by each complete rotation is about $_{1}/_{36}$ in. = to about 70 μ. This arrangement is exceedingly ingenious, for it will be observed that rapid working with high or low powers is brought about by the use of a *coarse* threaded screw (the threads being 36 per in.) that offers every reason for believing it will stand severe wear and tear.

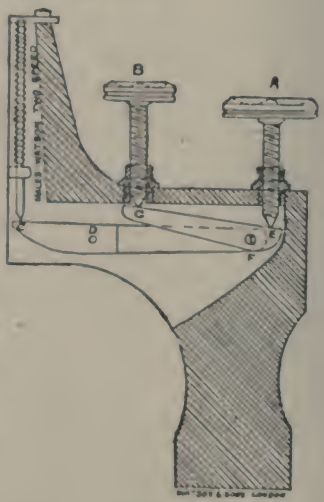


Fig. 57.

one to employ. Using a magnifying power of 1000 diameters, or anyhow one of 600, the central nodule of the Pleurosigma balticum should be focussed. In its floor will be seen two little balls. As the tube is lowered the first image shows these balls

By Carl Zeiss, Jena. (*Original Pattern.*)

In this arrangement (Fig. 58) B is the carrier for the microscope tube, fitted with rack and pinion, and O the prism on which the carrier B slides up and down. The spring P pushes on one end *towards* the nut E, which is tightened to the prism O, and on the other *towards* the tube carrier B, so that when the latter is forced towards E the spring will act in pushing it down again. The micrometer female screw F, as seen in the sketch, is screwed into the tube carrier B and fixed, whilst the nut E is provided with a hardened steel centre, on which the hardened steel point of the micrometer screw rests. If this screw is moved upwards, the spring P will push the tube carrier B down towards the stage of the microscope, which means that the objective is brought nearer to the object; whereas if it is screwed down it will raise the tube carrier B, pressing the spring P closer together. The screw K serves for the purpose of clamping the fine adjustment in any desired position, so that it does not move on account of vibration, keeping the object accurately in focus.

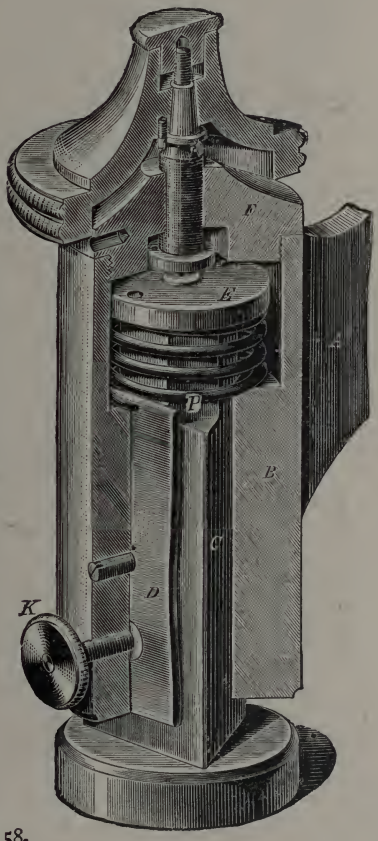


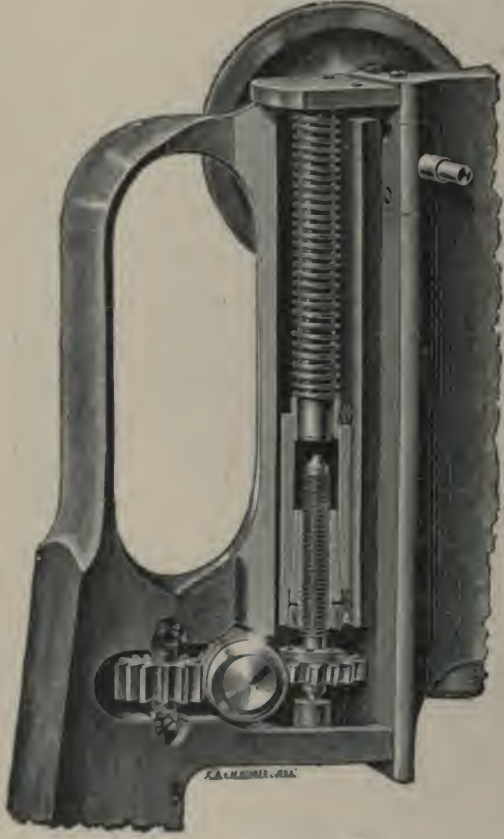
Fig. 58.

white, whilst an *extremely small additional lowering* turns them black. Set the focus with the balls white; tap the microscope and rap the table; no change to black should be visible after so doing. (See Figs. 1 and 2 in Plate I.)

However much the construction of the fine adjustment varies,

the mechanism is always to be found in the body of the instrument. This will be best seen by examining the figures representing the first-class microscopes that follow towards the end

By Zeiss. (*Latest Pattern.*)



A worm wheel, movable by means of an endless screw, is situated at the lower end of the micrometer screw, and serves in a sense as a screw head. The whole mechanism, with the exception of the two milled heads acting upon the endless screw, is enclosed inside the crane-shaped carrier, and is thus protected against all damage from outside. As the axes of the milled heads used in the fine and coarse adjustments are parallel, and as in elevating and depressing the tube both turn in the same direction, the change from one movement to the other is most convenient for the hand of the operator.

Fig. 59.

of this book, but the details of the fine adjustments by different makers are shown in Figs. 46–59 inclusive, a short description being given with each illustration. The description of the latest fine adjustment by Swift & Son will be found in the Addenda.

CHAPTER IV

THE OPTICAL PORTION OF THE MICROSCOPE

WHATEVER part may be played by the perfect construction and workmanship of a given stand, or the beauty and refinement of its different adjustments, still the real utility of the complete instrument taken as a whole must finally rest in the performance of the objective. This is of course true seeing that the separating power, the greater part of the magnification, and the beauteous details of the image of the object, all rest and are dependent upon its perfection. In saying this so emphatically, we do not wish it to be thought that we ignore the part played by the ocular, as if it had nothing to do with the building up of the final effect, for of course it has, especially with apochromatic objectives; but merely that its part is but a secondary one, for no eyepiece, however good, can ever overcome the effect produced by a bad objective. It is important then for the student to recollect this, so that if his pocket be but scantily filled, expense should not be stinted on the objective, whatever he may be compelled to sacrifice in the purchase of the stand.

The Objective—a collective term for numerous lenses placed together in a brass mount—is screwed upon the nosepiece of the tube, all combinations in the present day being so constructed as to fit the "universal fitting" already described when speaking of the mechanical portion of the microscope. The adoption by all opticians of this regulation size constitutes a very great convenience, seeing that it enables the microscopist to interchange objectives, whether English or Continental, without the necessity of employing supplemental adapters to suit the special requirements of each case, which in the olden days, as a matter of fact, was frequently unavoidable.[1] The objective, by

[1] The diameter of the objective screw-thread is ·8 in. The core thread is ·7644 in., the effective diameter is ·7822 in., the pitch is ·0277 in. The number of threads to the inch is 36—Whitworth form of screw. For complete specification and limits see Transactions of the Royal Microscopical Soc., p. 230, 1915. Sizing Taps and Dies may be obtained on application to the Secretaries of the Society.

means of its front lens, gathers the rays that, reflected off the mirror from the illuminant, have passed through the substage condenser on to and through the object, as shown diagrammatically in Figs. 60 and 61, which respectively represent an achromatic combination with a dry condenser, and an apochromatic objective and immersion illuminator. Emerging from the objective, it will be seen they enter the simple or compound field lens of the eyepiece, and, having passed through it, fall upon the next and last lens, called the eye lens, which transmits them to the eye of the observer.

It is not within the scope of this work to furnish the history and etiology, together with a description of the first conceptions and subsequent developments of the objective, but merely to speak of it as we find it in the present day. Constructively it is composed of at least two, but usually of several more, lenses held in a suitable mount of brass, which enables the combination as a whole to be affixed to the end of the tube of the instrument. The actual fitting for this purpose attached to the instrument is called the nosepiece, which, we have already explained, is bored to a definite diameter, and is cut with a screw thirty-six threads to the inch, furnishing what is collectively known as the Universal nosepiece or Universal fitting. By this arrangement it is obvious objectives of any make, whether English or foreign, can be used at will.

The components of the objective—the individual lenses themselves—are ground and polished out of glasses of differing densities possessing certain and various characteristics which need here be only mentioned; but it should be stated that, in addition, lenses of Fluorite, a curious mineral possessing very definite and unique properties, are interposed in the construction of the peculiar type of objectives invented by the late Professor Abbe, called his apochromatic series.[1]

The different little systems in a modern objective of the highest order are separated and mounted with the greatest care, the distance being in terms of hundredths, sometimes in thousandths, of an inch; hence it is important for the tyro to recollect not to meddle with the interior of any objective, as he

[1] Fluorite lenses are now often found in semi-apochromats. This peculiar substance has a comparatively *low* index of refraction and an *extremely* small dispersion. It is said that alum is almost as efficacious.

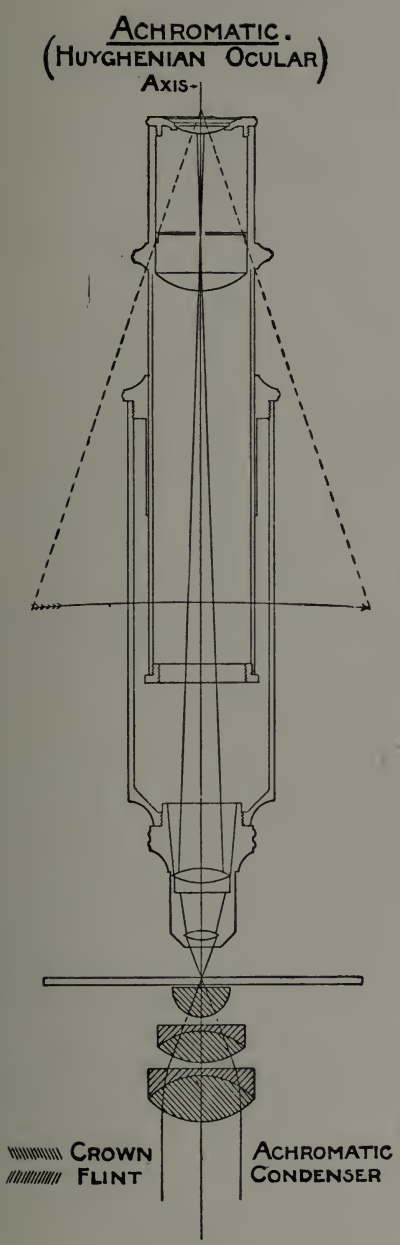

ACHROMATIC.
(HUYGHENIAN OCULAR)
AXIS
CROWN
FLINT
ACHROMATIC
CONDENSER

Fig. 60.

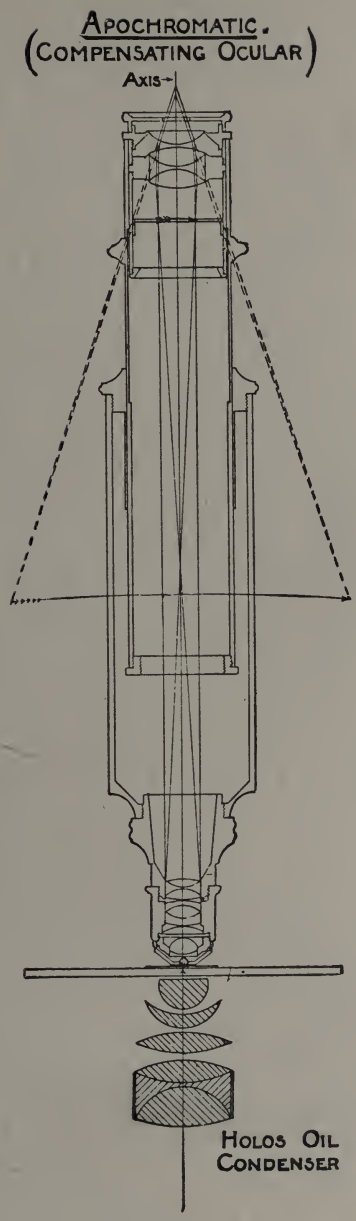

APOCHROMATIC.
(COMPENSATING OCULAR)
AXIS
HOLOS OIL
CONDENSER

Fig. 61.

may very easily upset its adjustments in a manner he cannot afterwards correct for himself. Especially careful should any user of the highest powers be that the front, which is nearly always, as we have already said, hemispherical—sometimes hyper-hemispherical—be not pressed upon or rudely treated; for to damage it, or even break it, is quite an easy matter, and occurs surprisingly quickly with but the slightest blow.

Objectives vary in their respective powers of magnification in direct accordance with their focal length, so some suitable means has to be adopted to distinguish the different kinds in use. Some opticians call their objectives by definite letters of the alphabet, or by the numerals 1, 2, 3, and so on; whilst others adopt a far better plan, that of affixing to them their focal length. This last-named method is decidedly the more rational and convenient, as the magnifying power can then be immediately known approximately, by dividing the conventional distance of distinct vision—10 in. or 250 mm.—by the focal length in question. When using either of the former nomenclatures this cannot of course be done, and if the objective's magnification be desired, the maker's catalogue has to be taken down and a search made for each objective, which is troublesome and unscientific. The precise magnification of an objective and how it is experimentally obtained is too long a matter to be entered into here; but it is fully discussed and explained later on in this work, as well as what is meant by its "working distance," which is a variable quantity even with those of similar focal length.

Every objective has to be corrected and adjusted by the maker for the length of draw-tube it is intended to be used with and with high powers, especially if "dry" lenses (a term explained at some length later), great care should be exercised that the draw-tube is set exactly correct, otherwise the performance of the combination is considerably upset, and it may be entirely ruined (see end of this chapter). Each objective is also corrected for a special thickness of cover-glass, usually about ·17 mm., and, if the cover be thicker, the *draw*-tube can be made to correct the error caused thereby by pushing it, with the ocular *in situ*, *nearer to* the specimen, the contrary direction being employed if the cover-glass be exceptionally thin. To be certain of the exact length of draw-tube being used, it is usual to find it graduated in parts of an inch or in millimetres, so that it can be set to

any length that the correction of the objective may require the microscopist thereby knowing the combination will be then placed under the most favourable conditions for its performance. After this is done, should the image not appear clear and sharply defined, it probably (if the objective be a good one) arises from the cover-glass not being of the same thickness for which the combination was corrected ; and, to alter this, the draw-tube is pulled out or pushed in, in the manner just described, which should at once render the performance as good as possible under existing circumstances.

Objectives are computed to fulfil three orders of condition, from which, indeed, their distinguishing names are derived—*achromatic*, *semi-apochromatic* and the *apochromatic*.[1] The first may be said now to be obsolete in the present day, excepting perhaps for very low powers indeed, like one or two inches ; for since the introduction of the Schott glass (often collectively called the "Jena glass") most opticians, recognising the new power placed in their hands, have recomputed their lens systems so that they now approximately fulfil the requirements of the next order of merit, called semi-apochromatism. The apochromatic series invented by the late Professor Abbe represents the consummation of theoretical and constructive skill, all wave-lengths coming to one focus, but owing to their necessarily enhanced price are not as commonly used for *everyday work*, being reserved for special occasions when the greatest possible definition or resolution is required, or when an object has to be photographed.[2] See Addenda, "Corrections required for different types," etc.

[1] It will be observed that no mention is here made of yet another type of objective recently introduced by the firm of Carl Zeiss, called the Monochromat. This is because the combination is not for use visually, but only for photographic purposes. The special feature of this system by Von Rohr is described as a very perfect union of rays (spherical correction only) for light rays of a definite wave-length chosen at will, the lack of correction for chromatic aberration being obviously because they are solely meant to be employed with monochromatic light. Such objectives are at present only manufactured to be employed for appliances arranged for the use of *ultra* violet light, their correction being made as a matter of fact for a wave-length 275 $\mu\mu$, all lenses being constructed out of molten quartz, the oculars for use with the same being of the same material, but out of the crystal form.

[2] We may explain in passing what we mean, by saying, that if an ordinary objective be used, and the object sharply focussed on the ground glass of the camera, the resulting image on the negative, taken in the ordinary

Then, too, in the study of diatoms, the apochromat holds its own to perfection, for the entire absence of all the colours of the secondary spectrum renders the image too beautiful to comment upon. Notwithstanding our saying this, however, we are bound to admit that Zeiss, Leitz, Koristka, Bausch & Lomb, and Himmler, not to mention Swift, Reichert, and Watson, have all recently manufactured semi-apochromats that furnish excellent images, and which, at the moment of focus, exhibit so little secondary colour as almost to deceive the very elect, and we heartily congratulate them upon the production of combinations of such excellent performance.

To demonstrate the theoretical difference between these three orders of objectives is not easy in a simple way: what follows, however, is an attempt to do so, being the substance of a Presidential address to the Quekett Microscopical Club upon the subject by the Author.

Before commencing, however, the reader should master (if he be not already acquainted with the subject) the few introductory paragraphs upon spherical aberration, coma, and chromatic aberration, for by so doing the subsequent remarks will be far better understood.

Spherical Aberration is a term which refers to a condition inherent in every uncorrected lens, by which is meant that

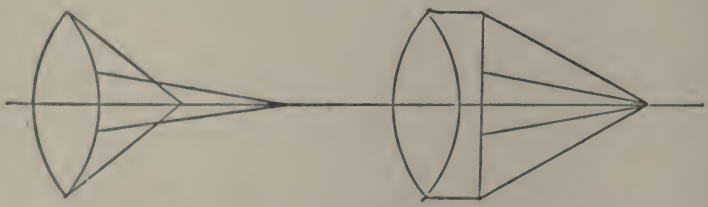


Fig. 62. Fig. 63.

the rays passing through its periphery, or outside portion, focus on the axis at a shorter distance than those passing through the more central portions of the lens, as shown in Fig. 62. If

fashion, will be found distinctly out of focus, because the chemical rays which act upon the photographic emulsion are not the same as those affecting the eye in focussing; whereas, if an apochromat be employed, the negative would appear practically as sharp as the visual image on the screen, because of the coincidence of the chemical and visual foci as just explained.

by suitable correction the rays are approximately brought to a common focus on the axis, the lens is said to be spherically corrected, as in Fig. 63 ; but if they are not enough corrected, although the foci are brought nearer each other than obtains in the uncorrected lens (Fig. 64, for example), the condition is

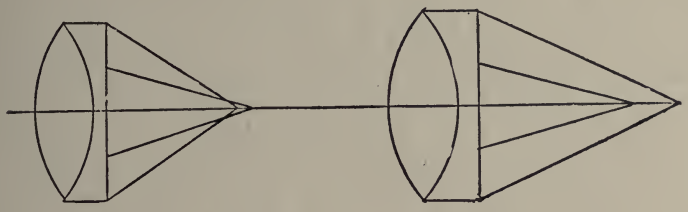


Fig. 64. Fig. 65.

spoken of as *under*-correction. In Fig. 65 we find just the reverse. The whole correction is too much, because the outer ray is taking the position previously occupied by the central ray, and the central ray that formerly occupied by the outer. As the correction is here too great, this state of things is called *over*-correction.

Coma is a defect confined entirely to rays coming from points of the image lying outside the axis of the lens, and one not easy to adequately define in what we may call simple language, for the reason that several who use the expression place upon it a meaning of their own. Although in what follows the expression is used in a somewhat restricted sense, still, even then, the nature of the fault is such that it is not easy to explain in a few words. To make what follows more intelligible, it will be best to hazard a definition first, that it is a peculiar defect due to the "non-fulfilment of the sine-law," because by so doing this will lead at once to an explanation what this sine-law is, and how its neglect causes coma.

In Fig. 66 the axis of the lens is shown at AA′, and L, a point of the object, is seen lying upon it. I is a ray proceeding from L to the lens O, meeting it at a point o^1, from which it passes to focus at F. Another ray following the course of II strikes the lens at o^2, focussing at the same point F. It is to be further understood, when in the future the zone o^1 is spoken of, it is not meant to include a piece of the lens from O to o^1, nor when talking of zone o^2, the portion

included between o^1 and o^2, but simply to refer to an extremely narrow belt of glass; so that, to make the meaning clear, a lens should be considered as being made up of a very great number of zones—like, in fact, a lighthouse lens is made—only two of which for the present purpose are being considered. The angle that the ray I makes with the axis AA′ on the left hand of the diagram we call α^1, that on the right side being designated β^1; whilst the angles for ray II are called α^2 and β^2 respectively for each side. Now the sine-law is this: that the ratio the sine of the angle α^1 bears to the sine of the angle β^1 shall be

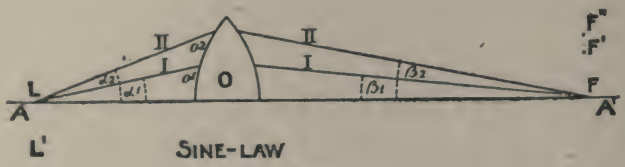


Fig. 66.

the same as the sine of α^2 bears to the sine of β^2. If, then, these ratios be equally proportionate, we speak of the "sine-condition," or the "sine-law" as being fulfilled. To make what follows more intelligible, let us, for example, say the angle α_1 measures 13° 54′, the sine of which ascertained by the tables is ·240; whilst the sine of β^1, assumed at 6° 55′, is ·120. Here it is evident the ratio is as 2 to 1. Extending the examination to the angles α^2 and β^2, let us further suppose they are 22° 58′ and 11° 15′, the sines of which are respectively ·390 and ·195—just the same ratio as before—viz. as 2 is to 1. This is what is called a "fulfilment of the sine-law." It has already been pointed out that coma is a defect confined to rays coming from points of the image lying outside the axis of the lens, but it remains to be said that the beauty of the sine-law is this: *if the sine-condition is fulfilled with regard to the strictly axial points of the object, it causes a distinct and good image of the portions of the object lying outside the optical axis*, such as we might say lies at L¹. Let us proceed, then, to see what happens if this sine-relation be neglected, and how such non-fulfilment causes the defect (whatever it may be) we call coma. To begin with, it should be mentioned the ratio between the sines, whatever it may be, is really the

magnification of the object with the lens in question under the circumstances, so that this particular lens we have been dealing with would magnify the object twice; or, to be more accurate, the zone o^1—that narrow strip of the lens containing the ray I—magnifies twice, and the zone o^2, containing ray II, does likewise. If, however, the sine-law be *not* observed by the computer of the lens, the effect is that these zones do *not* magnify the same, and hence that the rays coming from the outside points of the object will *not* focus on the same point; for ray I may come to a focus, say, at F′, whilst ray II is at F″.

In Fig. 67, the sine-law being supposed to have been fulfilled,

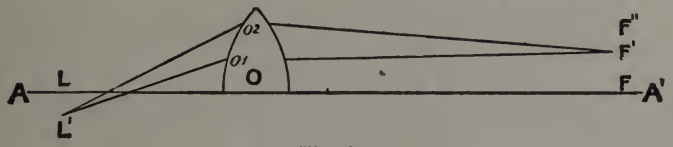


Fig. 67.

it is quite evident that the two zones are yielding exactly the same magnification, for they are seen to be both focussing at the same point F′; whilst in the next illustration (Fig. 68), the computer having neglected the sine-condition, the zones are shown coming to different foci—one at F′ and the other at F″. It is obvious how disastrous such a state of things would be for the definition; and if bad for two zones only

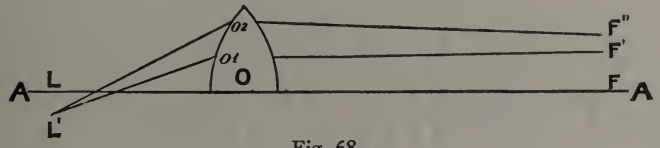


Fig. 68.

it can be easily imagined how bad it would be if all the zones of the object-glass were being considered. We should have a line extending from F′ to F″ instead of *two points*. But even yet the trouble is not at an end. This class of defect does not ever occur quite alone; other evil genii associate themselves, rendering "confusion worse confounded," their evil influences making their presence apparent by causing the line just spoken about to be spread out more and more into

something like a comet's tail: *this comet's tail appearance constitutes what is called Coma.* A curious effect probably due to either astigmatism or to the non-fulfilment of the sine-law is shown in two photomicrographs, Fig. 3, Plate III., and Fig. 1, Plate IV.

Chromatic Aberration.—This is another name for the errors which occur in the performance of lenses, in this case being due to the different paths pursued by light of *differing wave-lengths.* To simplify what follows, let us take first quite a simple lens, of short focal length—such, indeed, as met with in an eyepiece. Let us presume it is mounted in a cell, so that we can attach it to the nosepiece of the microscope, and that a piece of finely ground glass is placed over the eye-end of the draw-tube, the ocular having been first removed. A well-defined object being focussed on this glass screen—using different apertures of the lens in succession, and employing mono-chromatic light first of one colour and then of another—discloses the fact that, owing to spherical under-correction, we only get reasonably sharp images with a small aperture of the lens, and generally owing to coma, only in or near the centre of the field. If, with a small opening, we now try other colours of the spectrum in succession, we find that an alteration of the focal adjustment is required for each colour; and also that the different coloured images, when in focus with a constant tube-length, differ in size, for the red really requires a longer focal length and furnishes the smallest image, the violet having the shortest focal length, whilst it yields the largest image; other colours in between. This is better understood by examining Fig. 69. L is the simple uncorrected lens;

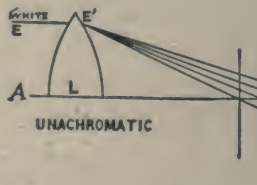


Fig. 69.

Fig. 70.

E is a beam of white light entering it; and E^1 the point where the lens, acting as a prism, breaks up the white light into its component colours. Red focusses on the axis AA′ at R, and violet at V, some of the other colours being omitted for clearness of

diagrammatic representation. This is the performance of the simple uncorrected lens. A great improvement is at once effected by combining a convex crown lens with a concave flint; the four radii and the different dispersive properties of the two glasses enable the computer to—

(1) Bring the central and marginal rays of one colour to focus at the same point—in other words, to remove spherical aberration for *one* colour, any wave-length that is chosen being spoken of as the "preferred colour";

(2) To correct coma for the same colour—in other words, to fulfil the demands of the sine-law for this preferred colour; and

(3) To cause the rays of other colours to concentrate very nearly upon the same point on the axis as those of the preferred colour.

The lens is now said to be achromatic in the ordinary sense of the word. If, however, the computer has over-corrected in the matter of colour, we shall have a state of things shown in Fig. 70, where red has assumed the shortest focal length and violet the longest; so we call this *total over-correction for chromatic aberration.* If we examine Figs. 71, 72, and 73 we

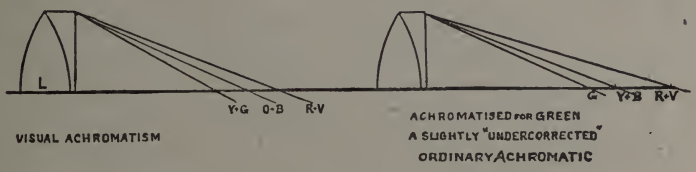


Fig. 71. Fig. 72.

shall quickly notice that the focal length of the preferred colour is always the shortest; so the spectrum may justly be said to be folded over at that point, for should the selected colour be yellow-green—which is, in fact, that usually taken for visual objectives—yellow-green will be seen to have the shortest focal length; orange and blue are folded together, and further on red and violet.[1] Achromatisation for green—what has been termed a slightly under-corrected ordinary achromatic—we see depicted in Fig. 72. Here the preferred colour has again the shortest focal length, a blending of yellow and blue coming next, whilst red and violet are seen still further on. A lens corrected for the

[1] The reader is especially invited to read the chapter on "Testing Objectives" which will make what has been said of more interest.

blue ray—an ordinary photographic objective—is drawn in Fig. 73. Blue is seen to have the shortest focal length, because it is the preferred colour; green and violet are joined up next; yellow and ultra-violet follow, whilst red, outstanding, has the longest focal length of all.

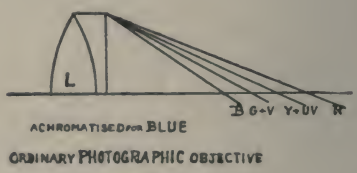


Fig. 73.

We shall find that in the best work, by making the components very thin, or else by very carefully proportioning their relative thicknesses, the computer can also produce, at any rate very approximately, equality of focal length for the different colours, which, it should be borne in mind, also means that the differently coloured images will be of the same size. Such combinations, when seen at their best, are said to have all the serious aberrations corrected in their first approximations. Indeed, such a combination, well effected, is near enough perfection if the objective be of small size and of relatively long focal length, say of not less than two inches; but directly the computer tries to make an objective of high power on these lines, where strongly curved lenses become necessary, a number of other imperfections soon become apparent, which rapidly place a limit on his powers. These secondary aberrations are as follows:

1. If we test an ordinary achromatic lens of considerable angular aperture, as previously detailed, with monochromatic light—the preferred colour selected being yellow-green for visual instruments, greenish blue for photo-visual purposes, and blue-violet for purely photographic work—we find that, although we have cured the spherical aberration of the central and marginal rays, by making them come to the same focus, the rays from the intermediate zones of the lens do not do so, being generally found to be under-corrected. This defect is often called "secondary spherical aberration," but, with the computer, "spherical zones." It is the most formidable of all troubles with which the optician has to deal, even in the case of objectives of moderate power; and it has to be corrected by combinations of over- and under-corrected elements acting in concert and in suitable sequence.

2. If we next substitute monochromatic light of wave-lengths

different from that of the preferred colour, we shall find that for them spherical aberration is *not* corrected, even in the first approximation, the defect being generally that of under-correction for red and over-correction for blue. This kind of secondary aberration is, in microscopical objectives, next in importance to the last mentioned, and has been called by the late Professor Abbe "chromatic differences of spherical aberration," which he further pointed out could not be cured without the use of glass of different properties from that which was then in existence. The outcome of this remark was the starting of the Jena glass factory.

3. If we proceed to test for differences of focal adjustment with the different colours, we find that whilst light of nearly the same tint as the preferred colour shows no sensible difference of focus, light of considerably different colour does, and in a somewhat curious fashion: If the lens be corrected for visual work, yellow-green, the preferred colour, will be found to have the shortest focal length, as we have before explained; the other colours, focussing at greater distances, blending, and in curious pairs, bright red being united with blue, and, at a still greater distance, deep red with indigo or violet, as we see in Fig. 71. This has been called the secondary spectrum, and is the most serious defect in telescopic objectives, but ranks only as an indifferent third in the manufacture of the microscopical objective. The earlier types of achromats made with the old kinds of glass will be found great offenders with regard to the correction of these secondary aberrations. In these the spherical zones are often found to be reasonably well corrected for the preferred colour, but the chromatic differences of spherical aberration are generally so serious as greatly to reduce the usefulness of this type—at any rate for accurate work, and particularly where a large cone of light is employed or oblique pencils are used. It is needless to say that for high-class photomicrography these lenses are simply useless.

As we have already said, the introduction of the Jena glass has changed the position of all things optical, for now we find the computer can produce objectives which are spherically corrected for two colours instead of only one, whilst the other wave-lengths are almost perfectly corrected; so we find ourselves face to face with an objective free from chromatic

differences of spherical aberration of which mention has just been made. These new objectives, if well made, should show no defect except that of the pure secondary spectrum. They appear in the market under many names—the achromatic of Zeiss, known as their "Improved Type," being the same as the semi-apochromatics of other makers. Sometimes we have entirely new names, such as the "Holoscopic" of Messrs. Watson & Sons, or the "Modern achromatic" of Messrs. Powell & Lealand and others. This new type is really a most excellent lens for all general purposes, but, of course, for photomicrography it requires the use of a suitable screen because of the secondary spectrum, and with diatoms the image shows colour. This secondary spectrum is entirely eliminated in the best type of apochromatic objective, which makes it rank as the highest achievement of modern computation, for besides the correction of spherical zones and "chromatic differences of spherical aberration," the secondary spectrum is *entirely* eliminated, leaving only the very faintest tertiary one outstanding. It should be further stated that as three colours[1] are united, instead of two as in the semi-apochromatic objective, photographs can be taken in light of any wave-length without alteration of focus or adjustment. If we glance at Fig. 74 the improvement is diagrammatically represented.

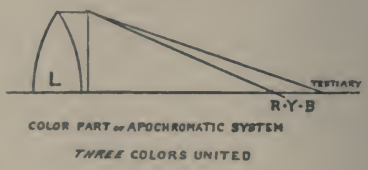


Fig. 74.

As all of these objectives necessitate the use of a special form of ocular, it may be well here to describe its function (for it is often misunderstood), especially as the use of a compensating eyepiece certainly improves the performance of the better class of semi-apochromatics.

Let us first try a high-power apochromatic with an ordinary Huygenian ocular, using white light with an object of strong contrast such as may be furnished by an Abbe test-plate. We find, perhaps to our surprise, excellent definition in the centre of the field, but an immense amount of primary colour (that is, yellow and blue) in the marginal parts of the field. No

[1] See page 365 concerning residual colours in apochromats, and Addenda concerning the magnification by different colours.

wonder, therefore, that even some experienced microscopists are led to think that the compensating ocular plays a very large part in the colour correction of this modern type of objective. But if, retaining the same ordinary ocular, we substitute monochromatic light of various colours in succession, we find, again perhaps to our astonishment, that without alteration for focus (or, at any rate, with but a very slight one in the case of the semi-apochromatic), any one colour gives extremely fine and sharp definition *all over the field of view*; but that the different coloured images are of different magnitude—red, for example, producing considerably lower magnification than blue or violet. This peculiarity is due to the thick un-achromatic front lens found in all these modern constructions, which causes them to have a shorter equivalent focal length for blue than for red rays as before explained. A compensating ocular, then, is merely one which has a corresponding chromatic difference of magnification *but in the opposite direction*, although of the same amount, so that the final magnification is the same for all colours. This chromatic difference of magnification is, then, *the only aberration that the compensating eyepiece can or does correct*: all the other corrections must be effected by the objective itself.

In conclusion, it may be of interest to tabulate the number of conditions which the objectives of various degrees of perfection fulfil, as this will show the difficulties the ambitious computer has to face.

(*a*) An ordinary low-power achromatic must be corrected for—

(1) Primary spherical aberration;
(2) Elimination of coma;
(3) Primary colour.

(*b*) An ordinary high-power achromatic, in addition, must be approximately corrected for—

(4) Secondary spherical aberration (spherical zones) for the preferred colour.

(*c*) Semi-apochromatics, besides fulfilling the above four conditions in a highly perfect manner, must be made free from—

(5) Primary spherical aberration for a second colour (and approximately for all colours); and

(6) Should be computed so as to give equal magnifications for all colours when used with compensating eyepieces ; whilst

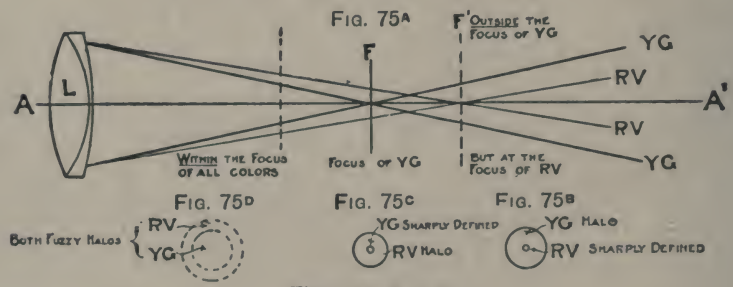
(*d*) The full apochromatic must further show—

(7) Freedom from the secondary spectrum.

In carrying out these corrections it is essential to bear in mind that the aberrations should be corrected in the order given ; an objective must be free from the first three to be entitled an achromatic, from the first five to become a semi-apochromatic, and from all seven to merit the true application of the term apochromatic. See Addenda.

Although scarcely in logical connection with the subject in hand, still it is of interest to add that a study of the following diagrams (Figs. 75A, B, C, and D) will furnish the reader with an explanation of the colours perceived when a high-power semi-apochromatic is employed upon such minute objects, for example, as diatoms and is lowered beneath the focus and raised above it. To make our meaning more clear it is convenient to deal with a point of light as shown in the figure.

AA′ is the axis and L the lens. The colours apple



Figs. 75A, B, C, D.

green (by the combination of yellow and green) and purple (by the admixture of red and violet) are only shown, the union of the orange and blue being omitted for clearness' sake. Yellow-green rays are seen coming from the lens and focussing at F, whilst the purple beams are uniting at F′ ; so that F′, whilst being the focus of red and violet, lies outside the focus of yellow and green. When therefore the objective is *raised* the focus F′ comes into view ; hence if a section were

possible to be made at that point, the centre, *sharply* defined, would be coloured with red and violet mixed, with yellow and green in union outside as a kind of halo, as shown diagrammatically in Fig. 75B. Pushing back the objective to the plane of F, the centre is still sharply defined, but the colour is changed to apple green, whereas the halo is composed of purple as shown in Fig. 75C; whilst lowering further, actually within the focus of the yellow and green (the visual focus already spoken of), we find a *fuzzy ill-defined centre* of apple green surrounded by a fuzzy halo of purple lying outside it as shown in Fig. 75D. It is obvious in Fig. 75B why the centre is sharp, because the position selected is the focussing point of the purple rays; and also why the centre is equally sharply defined at F in Fig. 75A, because that plane is the focussing point of the apple green; whilst the fuzzy appearance of both colours, apple green and purple, as seen within the focus (in the same figure), is owing to both images being out of focus in that situation. To witness the experiment really well with a microscope, a faint point of mercury on a slip should be used, but the phenomena are better seen with a telescope and a star.

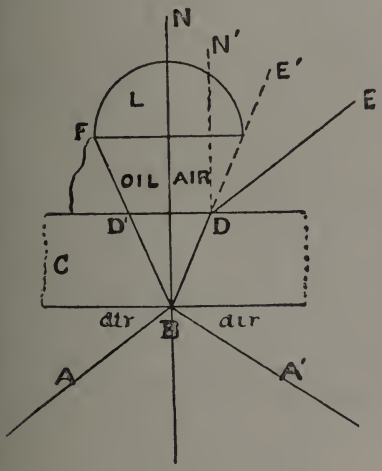


Fig. 76.

All objectives are divided into two kinds, whether they be achromatic, semi-apochromatic or apochromatic—called dry and immersion. To understand the explanation that follows, the reader, if a beginner, should refer back, and read what is said about light and its refraction until the subject of prisms is commenced. This being done, the following explanation of the difference between the two types of lenses will easily be grasped. Consider Fig. 76.

Let C be the *cover*-glass, having a refractive index of about 1·5, and L the front lens of the objective; also consider AB an incident ray falling upon the cover-glass at B. As it enters

a denser medium than air, according to the precept already laid down, it *must* be refracted *towards* the normal BN, and follow the path shown as BD. When it arrives at D (as in the case of what happens when using a dry lens) it passes again into air, a rarer medium, so is bent away *from* the normal DN′, as much as it has been bent *towards* BN, the previous normal, on entering the glass, thus continuing its course along DE to E; DE, therefore, is of course parallel to AB. It will now be readily understood, without much further consideration, that any rays lying between DE and the edge of the objective (where DE′ touches it in the figure) will be lost to the microscope, as they have no chance of entering the front lens of the objective.

But consider what happens if we place between the lens and the cover-glass a drop of fluid, say cedar oil, which has the same refractive index as the cover-glass—viz. 1·5. Following the diagram, commencing at the right hand, let the ray starting from A′ be considered. Arriving at B it will be refracted to D′ just the same and for similar reasons as AB was refracted to D. But as the emerging ray at D′ enters a fluid of the same refractive index as the substance it has left, it continues its path *uninterruptedly* in a straight line to F, which enables the object glass to gather up *the whole* of the rays that were lost when the ray entered air (as shown, for the sake of comparison, on the other side of the diagram) instead of the oil. *It is obvious then why the immersion system gives much more light.*

Theoretically, then, it is seen why dry objectives cannot pass so much light as immersion ones, because all the outside rays must be lost without the interposition of some homogeneous substance *optically* to join up the gap between the objective and the cover-glass. For this reason immersion lenses are often spoken of as "homogeneous systems."

We have already pointed out that as cover-glasses vary in thickness, so the bending of BD may vary in direct accordance, and it is to obviate this that dry lenses of high power are often provided with what is called "cover-glass adjustment," or "correction collar," which is so contrived that, by turning a milled portion of the mount, the lenses are separated or brought nearer together to accommodate the system to the

different thicknesses of the cover-glass mentioned.[1] It is well for the student to practise the use of this adjustment, and, to facilitate his learning the same, he is advised to obtain an Abbe test-plate (Fig. 4A, Plate I.) from Carl Zeiss, consisting of several cover-glasses (cemented on to a 3 × 1 slip) of stated thicknesses. This enables him to practise getting the best definition of the lines of silver deposit on any of the covers or all in succession, whilst he turns and "adjusts his collar" to the objective. When he thinks he has obtained the finest results, he then compares the reading on the collar with the figures on the plate, and sees if they approximately correspond, repeating the process if he is wrong, by which means he will learn to educate his eye to recognise a perfect adjustment, an art acquired only by constant practice. *Before commencing operations, it is to be recollected, the "draw-tube" should be set exactly correct for the tube-length for which the objective is constructed.*[2] It need scarcely be added, even if the objective be not provided with a collar adjustment, he will have valuable practice, and much experience may be gained in trying to obtain the best visual images of the lines of the plate by pushing in or pulling out the draw-tube as already explained. Unfortunately, however, the student, in this instance, has no means at his disposal of verifying his results, as we have just explained obtains when using a correction collar, by comparing the figures on the objective with those on the plate.[3]

[1] It may be interesting to those who like to know the details of the arrangement, to learn that moving a collar from, say, ten upwards to correct for extra thickness of cover causes an approximation of the other components of the system to the front lens of the combination; whilst turning in the opposite direction, of course, serves to produce a separation. *Approximating* the components, in the manner described, serves to introduce a small amount of *under*-correction in the system, which balances the *over*-correction caused by the extra thickness of a cover. Exactly the same correction is caused by pushing in the draw-tube when no collar adjustment is present. Some microscopists prefer doing this because, unless the collar adjustment be perfectly made, a certain amount of *de*-centring of the components is introduced, which interferes with the performance of the objective. See footnote, p. 78.

[2] By the length of the tube is meant the distance from the nosepiece at the milled shoulder of the objective to the upper end of the draw-tube.

[3] It should be recollected that although *usually* the figures marked on the "collar" are very approximately correct, still that occasionally a small error *may* be present.

One more point should not be overlooked. As the presence of the cedar oil forms a homogeneous system, so it is obvious any small variation in thickness of cover-glasses does not make so much difference when using homogeneous systems, hence such are not usually supplied with cover-glass adjustment collars, although later on we shall point out occasionally they are of service.

It should be borne in mind as an objective is composed of so many different lenses, the focal length it is said to possess does not relate to any one lenticular component, but to the combination *taken as a whole*, as if, in fact, it were a single lens of the focal length mentioned; hence the measurement of the distance at which a combination works from the cover-glass is not exactly that of the focal length, and we mention this because it is a mistake very often made by beginners, that they think a twelfth, for example, works at that distance from the cover-glass, whereas the real working-distance is between $\frac{1}{180}$ and $\frac{1}{200}$ of an inch instead! The computation of objectives by different opticians leads combinations to vary in this respect somewhat considerably, hence it should be further borne in mind that when the working-distance of an objective of given focal length is furnished by *one particular maker*, it does not follow at all it is the same for another (although of the same focal length) made by *a different optician*. It is a subject of regret that all firms do not give the working-distance of their objectives, for a long one is a great recommendation, and a matter often overlooked by the student who is making a purchase. The use of a long working-distance is particularly noticeable in laboratories, where the microscope is used as a tool and often not too carefully handled, for if such free distance be very short with the lens in use at the moment—a shorter one perhaps than that possessed by the objective more commonly employed and with which the microscopist is consequently more familiar—an accident may happen with surprising rapidity. It may occur *before* the object is focussed, or even before it can be indistinctly seen, owing to an unusually thick cover-glass. This, of course, arises because such cover uses up—so to speak—all the available free working-distance of the objective before it can come to a focus. To prevent an accident of this kind, special directions are given later on when dealing with "How to use the Microscope," which

should be carefully read by the student before attempting to use high powers (page 221).

Further, in consequence of very high-power oil-immersion systems requiring a more or less *hyper*-hemispherical front, it is obvious that such an objective is much more sensitive to accident by "shock," and consequently demands much more care in using, than one of lower power, where the front, being only *hemi*-spherical, permits of being so much more tightly held in its mount. This is very readily seen by examining Fig. 77 (after Zeiss). On the right hand is an objective of lower

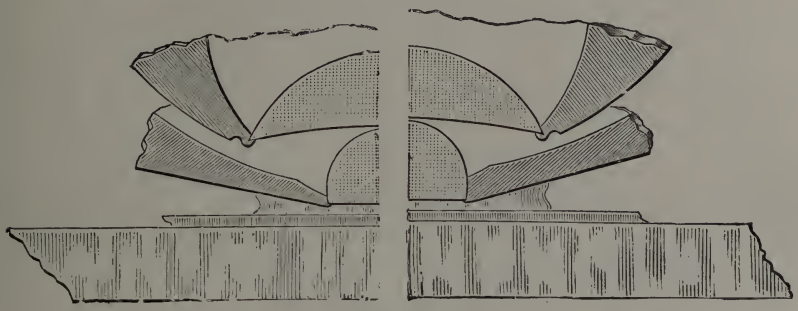


Fig. 77.

power than that shown on the left; the difference in the thickness of the "mount" in one case from that of the other is very obvious.[1]

We have previously said that, as a rule, no high-power homogeneous objectives—save some of those manufactured by Messrs. Powell & Lealand—are made with correction collars, for the reasons already given; but we feel bound to say that at times, on certain occasions, we have found the utility of using the correction collar with our Powell & Lealand apochromatic, and of recognising the greater convenience it affords of making the adjustments by this means, rather than by alterations of tube-length.

Throughout this chapter, and elsewhere in these pages, it

[1] As the term "numerical aperture," usually written N.A., has not been explained as yet, we refrain from introducing it in the text; but we may mention that the right-hand part of the diagram shows the mounting of an objective of N.A. 1·30, whilst the left is that of a combination with an aperture of N.A. 1·40.

has often been stated that objectives must be employed with the length of draw-tubes for which they are corrected, or otherwise they will not perform at their best, this being especially the case with dry systems; but no mention has been made (for fear of complicating the text) of the circumstance that combinations corrected for one tube-length can in certain instances be readjusted and arranged by the optician so as to work upon the other.[1] These can be more readily effected in the case of changing short-tube objectives into long-tube combinations than in the opposite manner, which will at once be understood when it is mentioned that the correction in the first instance merely consists in bringing the hemispherical front lens nearer to the other components of the system, which can be easily done by making the cell screw further on to the mount; whereas in the second it implies an increase in the length of the mount—often necessitating a new one—so that the cell bearing the front lens shall be removed a greater distance than before from the rest of the system.[2]

When finished with, all immersion systems should be carefully wiped surface-clean, and gently touched with a fine piece of cambric (moistened only) *with Xylol. Spirits of wine*[3] *must absolutely be avoided*, as in some instances it dissolves the cement holding the front *in situ*, and so do certain forms of cedar oil. The microscopist had better buy the immersion fluid sold by the maker of the lens. Of late, Mr. Nelson has contrived the mixing of a new immersion oil called "Mersol."

[1] See Index for reference to "Van Heurck's 'transformer.'"

[2] When using a sixth with collar adjustment, it may be at times useful to recollect that, seeing it is only necessary to separate the components sufficiently to convert a long tube into a short tube combination; so moving the collar adjustment backwards from, say, 18 to 10 or thereabouts (which brings about a separation, see footnote, p. 75) produces the required result. If this be not quite satisfactory, and the collar will turn no more, a slight alteration of the tube-length will be all that is required. *Vice versâ*, a change from the short to the long tube can be effected by reversing the order of things, viz., turning the collar from, say, 18 to 23, subsequently making any alteration in the tube-length that may be necessary.

[3] Particular attention is called to the matter of *not* using spirits of wine, as, unfortunately, in a book circulating amongst microscopists its author inadvertently recommended it to be employed!

CHAPTER V

NUMERICAL APERTURE AND DEPTH OF FOCUS

THERE are many who use this expression that find considerable difficulty in really understanding the comprehensive meaning of the term : continued observation and direct experiment have led them to recognise the fact that the greater the numerical aperture of a lens (usually written N.A.) the greater its resolving power, but beyond this they know little else. "Numerical aperture" is often looked upon by them as a mysterious term, the solution and thorough comprehension of which is a thing of great difficulty—only fit for experts—and one that is, they think, wrapt in mathematical clothing of a character far too recondite for them to understand. But in reality it is nothing of the sort ; still it is a difficult subject to explain intelligibly in a few words. A conscientious microscopical observer, however, one who is desirous of mastering his subject in its entirety, must not fight shy of the matter, for he will certainly require a complete knowledge of it whatever trouble it may cost him to acquire, if he wishes to use his objectives and microscope generally with judgment and intelligence so as to obtain the best results possible.

The first difficulty lies in the very word "aperture." Used in its legitimate sense of course it means "opening," and we speak, for example, of the object glass of a telescope being added to in aperture when its linear diameter is increased, and we know, too, that by so doing both its light-grasp and powers of resolution are greatly augmented. But what about the increase of "aperture" in the microscopical objective? The student naturally asks, How can this term apply, seeing that the higher the numerical aperture the *smaller* is usually the front lens of the combination? To this we must reply, before

offering explanation, that the term is applied to the "system," and not in respect to any special lens back or front. In the *system* of a telescopic objective the lenses are of about the same diameter throughout, so the increase of aperture of the system corresponds with the increase of diameter of the lenses, or *vice versâ*; but in a microscopical objective it is different. Increase of aperture here for the most part depends upon the increase in the ratio of the semi-diameter of the back lens to the focal length of the system, hence the diameter of the front lens before spoken of has nothing to do (in a sense) with the aperture question, and is only made of sufficient diameter to meet the requirements of the designer of the lens system. Thus far for the use—the restricted or special use—of the term "aperture," but no explanation has been offered why this increase in the ratio of the effective semi-diameter of the back lens to the focal length so improves definition, light-grasp, and resolution. To do this, we must first enter into the formation of the microscopical image.

In years gone by it was assumed that the image formed by the microscopical objective was dependent upon the same method of explanation as obtains with the telescope. It was thought that its formation was explainable in the same manner, and that the image of the delicate structure of a bee's wing in the microscope was formed by the same dioptric laws as govern the formation of the image of the sun or moon in the telescope; and that the image of the minute details of the most delicate diatom, as displayed to the eye on looking through the microscope, was geometrically traceable by applying the laws which govern the refraction of a ray of light, just in the same way and after the same fashion as could be shown to hold good in explaining the formation of the image of a distant view upon the ground glass of an ordinary camera by a photographic lens. In the present day this error seems to have taken a stronger hold than it otherwise would, because it has been found convenient by some exponents of the science to compare and explain the value of "aperture" in the microscopical objective by directly comparing it—without the necessary qualifications—with the "aperture" of the telescope. The comparison, we have said, properly understood, and so far as it goes, is good, seeing that an increase of the function in each case

improves definition, light-grasp, and separating power. But to the commencing student, as we have already pointed out, it is just this comparison, improperly qualified, that forms his first stumbling-block.

Having then asserted that the production of the image by these two instruments is not similar, to what then is the formation of the microscopical image due? Professor Abbe, whose profound mathematical knowledge was only equalled by his complete acquaintance with practical optics, was engaged for many years in vigorously attacking the problem, and he found that the perfection of the image produced by the microscopical objective was entirely dependent on the quantity of diffracted *light*[1] emanating from the object that was grasped by the objective and transmitted to the observer's eye. He proved his theory experimentally by means of artificial rulings on silvered glass giving regular diffraction *spectra*, and showed that when the number of these spectra admitted into the objective was diminished more and more by the insertion of suitable stops behind the objective, the definition and resolution of the rulings fell off more and more until eventually all traces of structure vanished when only one spectrum, or the direct light only, was admitted.

So far, then, the only point to be held by the reader is the fact the more diffracted light that can be *ad*mitted and *trans-*

[1] The rectilinear propagation of light on which the geometrical theory of optical instruments is based, and which is most directly exemplified in the formation of shadows, is only approximately true. So long as objects and apertures of fair size are concerned, the departure from rectilinear propagation is small; but when small objects or apertures are dealt with, and especially when their size becomes commensurable with the wave-length of light (equal to about $\frac{1}{50000}$ of an inch), it becomes quite obvious that they have a *scattering effect* on the light passing round or through them. Familiar examples are the appearances of a small flame when seen through a feather or through a piece of fine woven material such as a silk-handkerchief. Light which is thus scattered by small objects or apertures is called *diffracted light.* When a considerable number of similar objects or apertures are arranged at equally small intervals so as to form a *regular* pattern, the diffracted light assumes a still more striking appearance by being broken up into a series of isolated beams, which when derived from white light show the prismatic colours and are then called *diffraction-spectra.* All these appearances are easily and completely accounted for by the undulatory theory of light; they are treated of in Chapter XV., to which the reader is referred for further information.

mitted by the microscopical objective, the better the definition and the greater the resolution. It is obvious, then, the wider the angle embraced by the objective the better. This was known in olden days, and we shall speak of it again ; but the mistake then made was that the improvement in definition and resolution was thought to be due to the increase in obliquity of the rays from the object to the objective in high-angled objectives. This Abbe showed to be false, for he justly remarked a judicious tilting of the objective or specimen should do likewise, which on actual trial was found not to be the case. His studies led him further, for he found that the actual improvement did not take place as the angle of aperture increased, but as the *sine* of half that angle. This sine of half the angle, called sin U (where U is the half angle), he also conjointly proved, with another mathematician, was equal to the ratio of the semi-diameter of the emerging pencil, measured in the upper focal plane of the objective, to the focal length, which for simplicity and fairly close approximation may be said to be the same as the ratio of the semi-diameter of the effective aperture of the back lens to the focal length. This ratio for dry lenses is, we shall see, their numerical aperture ; but when we speak later of homogeneous systems, whose complete construction requires the uniting of the front lens of the objective to the cover-glass of the specimen by means of some fluid of proper refractive index, this special construction demands the introduction into the formula sin U of "*n*," the refractive index of the fluid employed, whether it be water, glycerine, or cedar oil. For convenience, then, it will be further shown this is the final formula of Abbe for numerical aperture of *all* lenses, whether dry or homogeneous, for it is evident such introduction in no way affects the validity of what has been said concerning the dry ones (as the index of air is 1·0, which therefore does not change their values at all), whereas its introduction into the formula enables all lenses, whether dry or homogeneous, to be directly compared, and places such comparison at once on a simple and scientific basis.[1]

[1] For those desirous of a mathematical proof, a consideration of the following argument in a concise form—for which I am indebted to Mr. Conrady —will be read with interest.

In Fig. 78 let L be a lens, or system of lenses, producing an image I of an object C. Let F be the point where the upper focal plane cuts the axis ;

Referring again to the diffraction spectra, it must not be thought they are imaginary or theoretical; they can easily be seen and studied without special accessories by focussing a high-apertured objective, say, one of N.A. 1·20 or 1·40, on

then, if CD is the marginal ray of the pencil which the lens is capable of receiving, EI the same ray after refraction, if α and β are the angles of

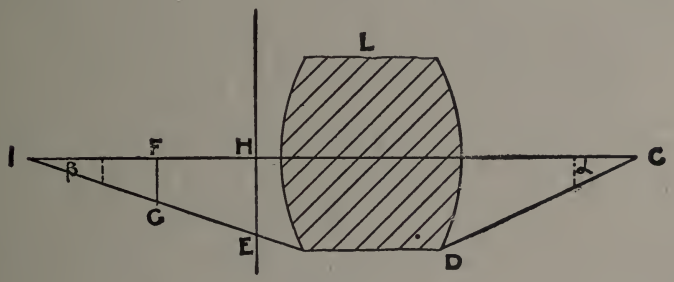


Fig. 78.

divergence before and after refraction, n the refractive index of the medium in which the object C is embedded, M the magnification of the image formed at I, and f the equivalent focal length of the lens or system of lenses, we have, by a fundamental dioptric formula,

$$\text{I. } FI = Mf.$$

In order to be aplanatic, the lens must fulfil Abbe's sine-condition, which demands—

$$\text{II. } n \sin \alpha = M \sin \beta.$$

Now β is always a small angle, and we may take its tangent as sensibly equal to its sine, or—

$$\text{Sin } \beta \simeq \frac{FG}{FI} = \frac{FG}{Mf}.$$

Introducing this value of sin β into II, we get—

$$\text{III. } n \sin \alpha = M \times \frac{FG}{Mf} = \frac{FG}{f}.$$

$n \sin \alpha$ is the numerical aperture of our lens, and FG is the semi-diameter of the emerging pencil taken in the upper focal plane, hence III forms the proof we require, viz.:

$$\text{N.A.} = \frac{\text{semi-diameter of the emerging pencil}}{\text{equivalent focal length}}.$$

That β is always a small angle follows from this: the back lens of an objective cannot be larger than $\frac{7}{10}$ in.; the tube-length is seldom less than 7 in. (with such a large lens), hence—

$$tg\beta \leq \frac{\cdot 35}{7} = \cdot 05, \text{ or } \beta \leq 2^\circ\ 52'.$$

For this angle the cosine—which is the ratio of sine to tangent—is about ·9987; near enough unity for purposes of N A. measurements. It is needless to remark the above proof includes any sort of objectives.

a Pleurosigma angulatum, and then, after removing the eyepiece and closing the iris diaphragm a little, by looking down the tube when a central beam of direct light will be seen surrounded with six diffraction spectra (Fig. 5, Plate I.). This has been tried on many occasions in pre-war times, by those anxious to prove whether the statement was correct or not, by obtaining the gratings as used by the late Professor Abbe (which were obtainable from Carl Zeiss), when it was readily recognised that the more these diffraction phenomena are cut off from entering into the formation of the image, so do the lines disappear from the grating used until at last, when the central dioptrically formed beam alone remains, all detail vanishes. Indeed, to use the words of Dr. Dallinger, "the image appears as if the objective has been removed and the microscope has lost its power altogether."

In this portion of the book it would be out of place to give these experiments in further detail, although they are referred to frequently elsewhere, but it may not be out of place to give the accredited summary of these classical experiments more or less in the Professor's own words:

"That an image produced by the microscopical objective of *a small object* is not geometrically produced *in its entirety* by the reunion of the rays of light emanating from different points of the object.

"That the greater part of the perfection of the final image is due to the diffracted rays that are collected by and passed through the objective, and not so much to the direct ones.

"That an image is *not* identical with the object unless the whole of the diffraction phenomena (which the object is capable of yielding) pass into the objective. The direct consequence of this is that if the whole of these phenomena be *not* received by the objective, then the image differs from the object according to the loss of the diffraction beams; in other words, the smaller the number accepted and passed by the objective the less faithful is the resulting image. When the detail in an object is coarse as compared with the wave-length of light, the whole of the diffracted light lies so close to the corresponding direct light that even low-power objectives will admit all that is of sensible brightness. Hence the correspondence between

object and image is very close. But when the detail becomes commensurate with the wave-length, or perhaps equal to a mere fraction of it, the diffraction spectra must be closely watched and the probable verisimilitude of the image estimated accordingly. Another point, too, is the fact that in the image formed of very small objects, such as two or more dots separated by a minute interval, the diffraction phenomena lie further out in the field of view from the direct rays than in the case of objects in which the details are separated by larger intervals, hence the greater importance still that the objective should have a high N.A. so as to enable it to catch and gather in as many as possible of these all-important rays." He further adds, speaking more especially with respect to details as easily "resolved" as in the case of Pleurosigma angulatum, that until an objective be made of N.A. 2·0 he cannot believe the final image (provided too, of course, that the slip, its cover-glass, and immersion medium have also the requisite index of refraction) can be regarded as an absolutely faithful reproduction of the original object.

But it is hoped the reader by now will understand why the increase of N.A. possessed by a lens (within limits to be hereafter defined) is of such importance, and see, too, the utility of giving to every objective the highest amount consistent with its initial magnification.

The evolution of thought is so rapid that the intelligent reader may now go a step further and very properly ask the following question. If the more the aperture we give to an objective the more its power of resolving, the greater its light-grasp and the more able it becomes to render a faithful image of the object, why do not lens manufacturers make their lower powers of higher N.A.; say, for example, why not make a half-inch with an aperture of 1·40, equal in point of fact to that of the best twelfth? At present the highest we know is that of Zeiss's ½-in. apochromatic and Watson's 12-mm. holoscopic, each of which has an aperture of about ·65. It is for this reason. First, supposing it were indeed possible to compute a lens of this description, it would demand such a large back lens that the combination could not be used on an ordinary microscope; and even if that were got over by using instruments with larger diameter of tube, the suiting of aperture

to power has a definite limit of usefulness.[1] Secondly, a normal eye of the very highest order is supposed to be able to resolve two objects—say two lines—about $\frac{1}{2500}$ in. apart when held at a distance of 10 in. With an objective of $\frac{1}{2}$-in. focal length and a 10 eyepiece, a magnifying power of 200 is obtained, which may be said to be beyond the limit of usefulness for an ordinary achromatic (as such an eyepiece would most probably cause what is known as a "rotten image"), but not overdoing the performance of a good apochromatic. With this magnification lines really $\frac{1}{50000}$ in. apart *appear to the eye in the microscope* as if separated by an interval of $\frac{1}{2500}$ in., and so would be capable of being seen by a normal eye; but closer lines than these, say at 70,000 to the inch, would be invisible, because the magnification would not be sufficient to separate them by an interval of as much as $\frac{1}{2500}$ of an inch, the

[1] It may be interesting as well as instructive for the student to consider the following remarks, as they will make him more conversant with the practical utility of what has been said. Seeing that the N.A. of a dry objective is practically the ratio of the semi-diameter of the available aperture of the back lens to the focal length, it is required to find the N.A. of a given dry objective whose focal length is $\frac{1}{3}$ in. and whose back lens measures $\frac{2}{3}$ in. across. Half the diameter then $= \frac{1}{3}$. We have now—

(1) - - - N.A. $= \frac{\frac{1}{5}}{\frac{1}{3}} = \frac{1}{5} \times \frac{3}{1} = \cdot 6$;

conversely, supposing a given objective of $\frac{1}{3}$ in. focal length is said to have an aperture of ·6, what must be the diameter of the back lens? Here we have—

(2) semi-diameter = focal length × N.A. $= \frac{1}{3} \times \frac{6}{10} = \frac{6}{30} = \frac{1}{5}$; hence the diameter $= \frac{2}{5}$ in.

Again :—an inch of ·3 N.A. would require a back lens $\frac{3}{5}$ in. diameter (because $(1 \times \frac{3}{10})\ 2 = \frac{6}{10} = \frac{3}{5}$). With a 10 eyepiece, the magnifying power being 100, such an objective should resolve three-tenths of the ideal lens of N.A. 1—viz. three-tenths of 95,000 lines to the inch, or about 28,500. If now the inch were used with a 20 eyepiece its magnifying power would be 200, the same as the half-inch with eyepiece 10, but to make it resolve 50,000 lines its N.A. must be raised, we have shown, to about ·6. But to do this by (2) the diameter of its back lens must needs be at least $1\frac{1}{5}$ in. : too large for any microscope. Further, if the half-inch were made of N.A. 1·40 as previously spoken of, it would require a back lens of, at least, $1\frac{3}{4}$ in. diameter and an eyepiece of 27 to be of any service; by which is meant, to equal in resolving power the performance of a $\frac{1}{12}$ in. 1·40 with 5 eyepiece, or a $\frac{1}{3}$ in. with a 7 eyepiece. It would then only just separate the lines sufficiently as to appear $\frac{1}{2500}$ in. apart in the field of view of the microscope—the limit of normal vision, and would require a microscope made for itself.

limit of the perception of the normal human eye. It is obvious, then, no more N.A. need be given to the half-inch than is sufficient *to effect the resolution required*—viz. 50,000 lines to the inch.

Professor Abbe has enunciated the law that twice the number of waves to the inch, of the light employed, multiplied by the N.A., furnishes the number of lines to the inch which it is possible for any lens to show when used with the assistance of oblique light, visually. Presuming the mean wave-length of visual light in air is about $\frac{1}{47500}$ in., then for N.A. 1·0 the number of lines to the inch capable of being resolved is 95,000. Proportionately, then, for 50,000 the N.A. required is about ·53 when using oblique light (to be hereafter explained), but more is required for what is called central illumination, the standard fixed being usually about ·65 for the half-inch apochromatic. It is obvious now that giving a greater aperture to the half-inch would be of no practical use: potentially the power of resolution to a greater extent would be *there*, but the eye could not utilise it or even recognise its presence. Seeing, however, the achromatic will not admit of the use of such high eyepiecing as ten diameters without producing a "rotten image," it is obvious that it is not desirable or indeed of any use making their N.A. so large as that of the apochromatic series of lenses. This is as much as saying that the resolving power of an achromatic half-inch is not so great as that of an apochromatic. Recently, however, the objectives made by Leitz, Zeiss, and others, notably those sold by Messrs. Watson & Sons under the name of the "Holoscopic Lenses," from computations by Mr. Conrady, are so perfect that they *do* admit of much higher eyepiecing than was borne by the older make, some indeed bearing almost as much as the apochromatic. For this reason Mr. Conrady has given Holoscopic lenses much higher N.A. than usual, so as to render their performance the more complete.

This ideal construction is not so very difficult to the able computer with low and medium power lenses, but with high-power *dry* lenses such as a $\frac{1}{8}$ in. or $\frac{1}{12}$ in. it is impossible. How the difficulties are got over by the introduction of the so-called Homogeneous or Immersion system and upon what their superiority depends, must be our next subject.

Before the enunciation by Professor Abbe of these ideas as to

the true formation of the microscopical image, opticians, as we have previously remarked, basing their belief that wide-angled objectives owed their superiority in definition to the *angle of obliquity* at which they could receive the rays from the object, and not being aware of the part played by the diffraction spectra, had religiously striven for years to increase this aperture angle of which we speak. They found, too, that an increase of efficiency was produced by continuing the *optical* continuity between the front lens and the cover-glass of the specimen by the interposition at first of water, then by glycerine, but finally and most perfectly by cedar-wood oil, because its refractive index was the same as that of most cover-glasses. But *why* this was so, and how such improvement was brought about, remained only a matter of surmise and one that led to a great deal of difference of opinion. By patient practice and computation with the glasses then available they had brought up the receiving angle of the dry objective to what appeared its final limit, viz. to about 170°; and what more, they said, in this direction could be expected or made?

Yet the fact remained as time went on that the oil immersion gave such improved results! Why was it? And to what was it due? Here indeed was a situation which seemed of insuperable difficulty. Some thought one thing and some another. Either the dry objective did not, after all, gather the rays at about 170° as their computers declared, or else the oil-immersion objectives must gather rays at a greater angle than 180°, which was absurd on the face of facts.

To understand this in a practical way and without any mathematical environment we must digress for a moment upon the subject of the simple refraction of light so as to enable the reader to follow what comes after and understand the subject intelligibly.[1]

Let A, B, C, D in Fig. 79 represent the outlines of a circular vessel, AC being the water line, and BD drawn at right angles to it passing from B to D through E, which direction is called

[1] This portion of the subject has really been furnished before in the earlier part of this work, but it is interpolated here to save the reader the annoyance of having to refer back at some length before he can continue with the present argument.

the "perpendicular" or "normal," all angles being referred to this line.

When the beam is incident along B to E perpendicularly into the new medium, there is no refraction—the only instance where it undergoes no bending—for it passes on into the water, uninterruptedly following the course of the line ED. But when it is incident at any other position—say at *m* along *m*E—there is refraction at E, for the beam will be found to strike the point *n*. Suppose it is incident at *m'* along *m'*E, then there is also refraction at E, for the ray will be found at *n'*.

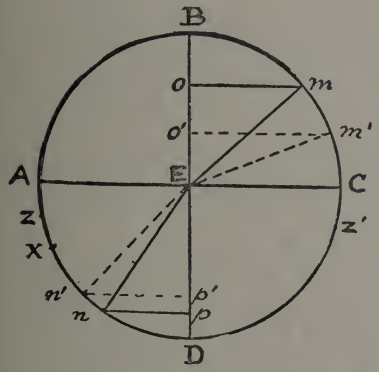


Fig. 79.

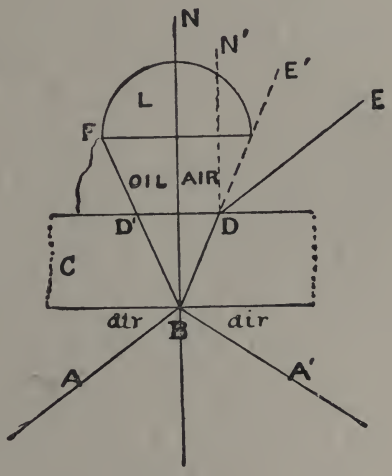


Fig. 80.

Snell made a celebrated investigation concerning this bending of the rays, by which their path can be predicted. Were it not for his discovery, about to be explained, we should not have had the grand computations of lenses with which, in the present day, we are all so familiar.

He first drew a line from *m* to meet BE at right angles at *o*, and another from *n* meeting ED at *p*. The lengths *om* and *np* were measured and divided one by the other; a quotient was obtained. Now what he discovered was the fact that wherever the angles were taken, whether from *m* or *m'*, the quotients, in all cases using water and air, came out the same, viz. 1·333. This was called the Refractive Index of water.

Other substances were tried, and each substance had its special refractive index. Flint glass, for instance, is found to be about 1·54 to 1·64, according to its manufacture, and so on with other substances, complete lists being found in all books upon the subject. If the reader be mathematically inclined, he will at once see these lines, *mo*, *np*, really represent the sines of the angles BE*m* and *n*E*p* respectively, so that, continuing our precept, the sines bear certain definite ratios one with the other wherever the incident light striking E comes from; that is, the ratio between *om* and *np*, which is about 4 to 3, holds good, whether the ray starts from *m* or *m'*. It is quite evident now that we can calculate where the ray will strike AD, after starting from any given point in BC. For example, let *m* strike E to make an angle *m*E*o*, say, of 45°. It is required to find the angle *n*E*p*, so that we can draw *n*E correctly. We take out of the tables the sine of 45°, and find, roughly speaking, it is 0·7, and multiply that by 3 and divide by 4, which gives us 0·5. Resorting to our book again, we find 0·5 is the sine of 30°, so that 30° must be marked off from D to find the line *n*E. Although, simply put, this is the maxim which mainly pervades the optician's mind in constructing new lenses, as a matter of fact the details become exceedingly operose in real calculations, as different colours are refracted at different angles, and the problem, where many lenses are concerned, becomes intensely intricate. But the law underlying the calculations is the same from beginning to end. The same law, reversed, of course, equally holds good when rays pass from the water into air, and when passing from one glass to another, although then with certain modifications.

One more remark on the subject has yet to be made. Seeing that *n'* passing to E becomes refracted to *m'*, what happens to a ray starting about *x*? It will pass into the air and graze along EC. If this be true, what will take place if one starts still nearer A, say at *z*? This ray cannot get out of the water at all, and is said to suffer "total reflexion" at E, for it appears again at *z'*. There is one angle then, it is very evident, which is the last, that allows a ray to get out; this is called the "*critical*" or "*limiting angle*," and is known for all kinds of glass.

Let us now see how these remarks apply to our subject.

Consider Fig. 80. Let *c* be the cover-glass, having a refractive index of about 1·5, and L the front lens of the objective. Also consider AB an incident ray upon the cover-glass at B. As it enters a denser medium than air, according to our precept it must be refracted towards the normal BN and follow the path shown as BD. When it arrives at D (as in the case of what happens when using a dry lens) it passes into air again—a rarer medium—so is bent away from the normal DN′ as much as it had been bent towards BN, the previous normal, on entering the glass, thus continuing its course along DE to E; DE, therefore, is of course parallel to AB. It will now be readily understood, without much consideration, that any rays lying between DE and the edge of the objective (where DE′ touches it in the diagram) will be lost to the microscope, as they have no chance of entering the front lens of the objective.

But let us consider what happens if we place between the lens and the cover-glass a drop of fluid, say, cedar oil, which has the same refractive index as the cover-glass itself, viz. 1·5. Follow the diagram, commencing at the right hand, and consider the ray starting from A′. Arriving at B, it will be refracted to D′ just the same and for similar reasons as AB was refracted to D. But notice now what happens. As the emerging ray at D′ enters a fluid of the same refractive index as the substance it has left, it continues its path uninterruptedly in a straight line to F, which enables the object-glass to gather up the whole of the rays that were lost when the ray entered air, as shown the other side of the diagram, instead of the oil.

This gathering up of the rays, which in a sense may be compared with the bunching up when light enters a denser medium from a rarer one, leads to a digression for a moment to explain certain difficulties which present themselves to the mind of the student, owing, we think, to text-books not being so explicit as they might be upon this subject, which is difficult to understand unless carefully explained in detail from commencement to end.

Starting from the beginning, one of the most remarkable features of the ether waves is their extreme rapidity of motion; another being that colour effects, as seen by the eye, are alone dependent upon the number of such waves entering the eye in a

second of time. Red light is caused by about 400 billions and violet light by about 678 billions of these waves entering the eye and striking the retina in that interval. As light travels in air 180,000 miles a second, so we calculate that the wave-length for red light is about $\frac{1}{39000}$th and that of violet about $\frac{1}{64000}$th of an inch in length. But in denser media, however, such as glass or oil, light travels slower, and as the number of waves for each colour is an unchangeable quantity, so the slower it moves the shorter the waves become, but the colour remains the same. It is just here that a puzzle often presents itself to the beginner, for text-books frequently lead him to understand that changes in *colour* are produced by *any* alteration of wave-length *per se*, instead of *only* by changes in the number of waves entering the eye in a second. Bearing this in mind, it can now be understood why no colour alteration is caused when light traverses lenses of differing refractive index, films of oil or such like; or when bunched up in immersion systems, the number of waves remaining *constant*, the *colour continues the same*.

Seeing that refractive index may be called the ratio of the velocity of light in air to that in any given substance, so the actual alteration of wave-length in different media is found by dividing the wave-length in air by the refractive index of the new medium. Thus D light is ·59 μ wave-length in air, but in dense flint of index 1·667 it becomes shortened to ·35 μ.

It now becomes evident, without further explanation, why this "bunching up" in the immersion systems gives to them so much more light chiefly due to diffraction which in turn improves both the seeing and the definition. It is equally obvious, too, as more rays are admitted by the immersion systems than is possible with the dry ones, that the latter can never compete with the former.

Lastly, a small consideration will show the difficulty that arose after the introduction of these immersion systems of comparing their relative merits. The "angle of aperture" as formerly used would not apply, because, as a matter of fact, the "*angle*" spoken of in one case, as in air, for example, is not of the same nature as the "*angle in oil*." They could never be compared. Continued misunderstandings were now for ever arising in comparing the performance of the different objectives —the dry, the water, the glycerine, and the cedar-oil immersion.

A brilliant thought, however, occurred to Professor Abbe, that if the refractive index of the fluid was taken into consideration—whether air, glycerine, or cedar oil—dry objectives could be at once compared with immersion systems in a scientific manner. Calling the refractive index *n*, the equation became *n* sin U, instead of sin U only, and this he termed the *Numerical Aperture of the objective*. For air *n* is only 1, for cedar oil 1·52, and so on. By this ingenious idea, then, comparisons of all objectives could be made at once upon a true and scientific basis.

Take for example:

A dry lens of 60° has a N.A. of ·5, because—

$$n \sin u = 1 \times \sin \frac{60^\circ}{2} = 1 \times \sin 30^\circ = 1 \times \cdot 5 = \cdot 5 \text{ N.A.}$$

Again, an oil immersion of 38·5° angle has a N.A. of ·5, because *n* (the refractive index of cedar oil) = 1·52:

$$\therefore 1{\cdot}52 \times \sin \frac{38{\cdot}5^\circ}{2} = 1{\cdot}52 \times \sin 19{\cdot}25^\circ = 1{\cdot}52 \times {\cdot}329 = {\cdot}5 \text{ N.A.}$$

Thus it is readily seen that a dry objective of 60° angle is equivalent to an oil immersion (where $n = 1{\cdot}52$) of 38·5° angle, because each one has a N.A. of ·5.

If we wish to compute problems in the opposite manner, we may suppose a dry objective to be ·5 N.A., and wish to find its angular aperture. Because *n* sin *u* = ·5, therefore sin $u = \frac{\cdot 5}{n}$. The objective being a dry one, $n = 1$, therefore sin *u* = ·5. Now opposite ·5 in the Tables of Natural Sines is seen the angle 30°, hence U = 30°. But it must not be forgotten that U is the half of the angular aperture, hence the aperture is really 2U = 30 × 2 = 60°, the angular aperture required.

Once more. An oil immersion has a N.A. of ·5: required its angular aperture. Here $n = 1{\cdot}52$ and *n* sin *u* = ·5, sin $u = \frac{\cdot 5}{1{\cdot}52}$ = ·329; therefore *u* = 19·25 (which is found by the tables), and 19·25 × 2 = 38·5°, which gives the result required.

From what has been said, it will be now understood the essential qualities of an objective that depend on its N.A. are:

1. *Brightness of Image:* this increases with a given magnification—other things being equal—as the *square* of the aperture.
2. *Resolving and Defining Power*—in direct proportion with the N.A.; whilst it will be seen later on that—

3. *The Depth of Focus* is intimately associated with the amount of N.A. present (but in company with other factors), being inversely proportional to it.

Ascertaining the N.A. of an Objective.

There are three methods for dry objectives, and two for homogeneous systems.

The first, applying to *both* types of lenses, is by using a special apparatus devised for the purpose by Professor Abbe, called the Apertometer (Fig. 81). This consists of a piece of thick glass about 3 in. in diameter, and half an inch thick. The part where the glass becomes segmental is bevelled from

Fig. 81.

above downwards to an angle of 45°. Near the centre (marked *a*) is a small disc of silvered glass with a small hole in its centre, where the silvering is removed. Two plates of metal, which can be shifted around the outer edge of the glass shown at *bb*, are square one side and pointed on the other (see *b* in the side view). To use this apparatus, the glass is placed with the graduated surface uppermost upon the stage of the microscope (fixed vertically) in such a manner that the circular portion is forwards, and the chord or bevelled piece backwards, towards the stem of the instrument. The edges of the little hole are now focussed with the objective to be measured, an eyepiece being used, and the length of the draw-tube the same as when the objective is ordinarily in use. The two indices, as *bb* are called, are then placed on the edge of the glass, as shown in

the plate, but close to the middle of the semicircle. Their sharp points should lie along the vertical edge of the disc, and their flat sides upon its upper graduated surface. It is best to direct the points away from each other to the outer side, if the power to be examined is comparatively high (N.A. above 0·6 or 0·7), but towards each other to the inner side if a low objective is employed.

With each apertometer an auxiliary objective is supplied which screws, or must be made to screw, into the end of the draw-tube, after which both are returned to the microscope, with the auxiliary lens passing down the main tube. The same eyepiece is then placed in the draw-tube as before, and the auxiliary microscope thus obtained is made to focus the image of the indices *by sliding the draw-tube in the main tube.* Care must be taken both in pulling out and pushing in the draw-tube for this purpose, whilst the eye is looking into the ocular, *not to alter the adjustment of the objective under examination* by accidentally shifting the main tube.

The indices are now adjusted, taking care they lie close to the glass plate, until their sharp points just touch the periphery of the luminous circle seen on looking down the eyepiece. Their position found, the readings of the upper edges, which lie in the same verticle plane as the points, are read from one of the two scales on the plate. The half of the sum of the two readings on the outermost scale—that nearest the edge—will give the measured value of the N.A. of the objective under examination. Likewise the sum of the two readings on the inner scale will give the value of the angular aperture in air.

The illumination must be shifted from right to left or up and down, so that the light falls horizontally upon the edge of the glass.

It should be noted that if the apertometer be used on *low*-power objectives, such as an inch, *with high N.A.*, owing to the size of the back lens having to be so large, the *auxiliary* combination may not be of sufficient diameter to give the maximum N.A. of the objective under examination. Also, and this is commoner still, with medium powers, say $\frac{1}{4}$, $\frac{1}{5}$, or $\frac{1}{6}$, it is not at all improbable the ordinary eyepiece, whether achromatic or compensating, may not command sufficient field of view; so

between the two troubles a false N.A. may be obtained. This actually happened in our case when testing an inch N.A. ·3, an apochromatic quarter-inch, and an achromatic sixth, the mistake being only discovered when applying as a check one of the following methods about to be described, when different results were obtained. To remedy the first fault with low powers, let the observer look down the microscope after the first focussing, and regulate the indices *without* the auxiliary lens, using no eyepiece at all ; whilst to avoid the latter trouble it is best by far to employ an ocular having no diaphragm on all occasions.

The student should read some interesting suggestions made by Dr. Hartridge upon the use of the apertometer contained in page 337, Part 4, *Journal Roy. Mic. Soc.*, 1918.

Another method of ascertaining the numerical aperture is by the use of a device suggested by Mr. F. J. Cheshire, now Professor at the Imperial Institute, South Kensington. This simple and yet efficient contrivance is described in a paper by the inventor in the *Journal of the Quekett Microscopical Club*, Vol. IX. (1904), to which the reader is referred for mathematical and other details. Briefly, the apertometer consists of a number of concentric circles, drawn on paper, which are so spaced and graduated in thickness that when the diagram which they form is placed on the stage of the microscope, at a certain distance below the usual object plane of the objective to be tested, the circles project into the upper focal plane of this objective, as a number of equi-distant, equi-thick concentric circles, from an observation of the number of which visible, the N.A. is read off directly. The first and smallest circle corresponds to an N.A. of 0·1, the second to 0·2, and so on up to 0·9. Intermediate values are estimated by the eye. The observation of the image of the diagram in the upper focal plane is made either by (1) removing the eyepiece and fitting the top of the draw-tube with a small peep-hole ; or (2) by fitting a low-power eyepiece—a 50-mm. Leitz does admirably—with a 2-mm. stop and using it to project the apertometer image in the eye-ring or Ramsden circle of the microscope, in which circle it can be observed with the aid of a high-power pocket magnifier. The draw-tube may be also used as an auxiliary microscope (as in the Abbe apertometer) by fitting its lower end with an objective suitably stopped to give a telecentric system.

The simple form of this apertometer, shown in Fig. 82A, is sold by Baker of Holborn; a modification (Fig. 82B), in which the circles are drawn on the lower surface of a thick disc of highly refractive glass adapted for use with dry and immersion objectives, is sold by R. & J. Beck of Cornhill, London.

With dry powers only, there yet remains another method which is also exceedingly simple and effective.[1]

Lay upon the table two pieces of white paper, using a black

Fig. 82A.

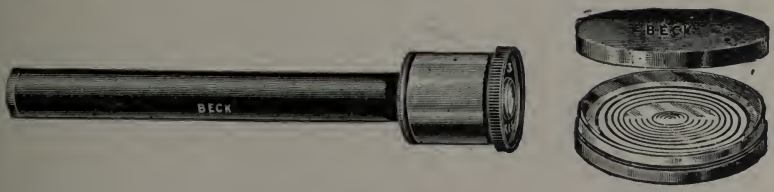


Fig. 82B.

background, with their straight inner edges parallel to one another and a definite distance (say 20 cm. for lenses of N.A. over 0·50, less for low-angled ones) apart, then hold a rule vertically upon the table about midway between the two pieces of paper. Next hold the objective to be tested vertically

[1] Although this idea may have occurred to others for obtaining the *angular aperture* of microscopical objectives, we believe it was first suggested to obtain their *numerical aperture* by Mr. Conrady, now Professor at the Imperial Institute, South Kensington, being published in the Author's *Photomicrography*, from which the paragraph is abstracted.

against the rule and look down at the back lens. Images of the two pieces of paper will be seen there: now slide the objective downwards along the edge of the rule, always watching these images. They will separate farther and farther apart until at last a point is reached where only a slight bluish flicker remains visible on either side in the extreme margin of the lens, which, of course, indicates that the inner edges of the pieces of paper are in the direction of the most oblique rays which the objective is capable of receiving, or that the angle enclosed between these directions, which directions intersect in the principal focus of the objective, is the angle of aperture. To determine this angle, read off the distance from the table to the front of the objective, and subtract the working distance of the lens,[1] so as to get the distance from table to focus. Then this distance divided by half the distance between the two pieces of paper is the cotangent of the semi-angle of aperture; the latter may, therefore, be obtained from a table of trigonometrical ratios, and the sine of the same angle is the N.A. of the objective.

Example:

Distance between the two pieces of paper, 200 mm.
Distance of front lens of objective from paper, 33·0 mm.
Working distance of objective, 0·2 mm.;

$$\therefore \frac{33{\cdot}0 - 0{\cdot}2}{200/2} = \frac{32{\cdot}8}{100} = {\cdot}328.$$

0·328 = cotan. of angle 71° 49′, as we find from the trigonometrical tables, the sine of which = 0·95 = N.A.

With great care this method will give results accurate to one or two units of the second decimal. In looking at the back of the objective the eye should be at a distance about equal to the tube-length for which the objective is designed, but the error caused by even considerable deviations from this theoretically required distance, is very small.

Depth of Focus

Before concluding this chapter, seeing that the amount of "depth of focus" (sometimes called "penetrating power") possessed by an objective is largely though by no means entirely

[1] How to ascertain the working distance, see Index.

associated with its numerical aperture at the time of use, the following remarks may be read with interest.

First, what is meant by the term? It is this. When the superficial part of an object is in focus, how much of its third dimension—that of depth—is sharp and well defined at the same moment? The subject is a somewhat difficult one, and not very easy to explain, for it was wrapped in obscurity until Professor Abbe took the question in hand, being up to that time thought to be an inherent and distinctly mysterious property possessed by certain objectives over and above that to be found in other combinations.

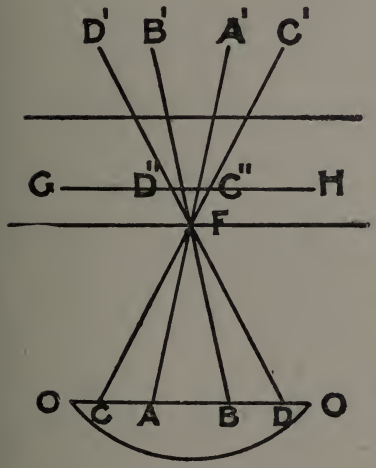


Fig. 83A.

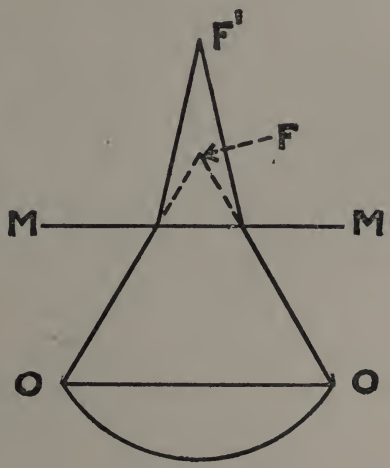


Fig. 83B.

As we have just said, to explain the whole subject intelligibly is not at all easy, and we feel a pleasure in acknowledging very considerable assistance from Mr. A. E. Conrady, who has given very much attention to the matter. To simplify what follows let us consider Fig. 83A, where an objective OO is focussed on a plane containing F. Supposing the aperture of the objective to be reduced to AB, then it is obvious that light from all points of a lower part of the object between A′ and B′ would pass through this point F and would be mixed with the image of this point; hence the confusion owing to the lower portion of the object being out of focus will be equal to the distance

from A′ to B′. Suppose we now increase the aperture of our objective to CD, then by the same reasoning we find that portions of the lower part of the object extending from C′ to D′ will now be confused; and it is easy to see that for a given distance between the sharply focussed plane and some other plane, the diffusion of focus for the latter must be directly proportional to the diameter of the object-glass and with sufficient approximation to the numerical aperture employed. But the above figure also makes it clear that with the larger aperture CD there is a plane GH much nearer the sharp focus F where the diffusion C″D″, corresponding to the large aperture, is the same as that (A′B′) produced by the small aperture at a greater distance from F. Hence we may also say that for a given allowance of diffusion the depth of focus is inversely proportional to the N.A., and in this form the influence of the N.A. will enter into our formula.

The effect of magnification is obvious. The dimensions of A′B′, C′D′, etc., are seen magnified by the microscope; hence the greater the magnification, the less the size of the diffusion A′B′ in the *object* which can be tolerated in the magnified image; the depth of focus must therefore be inversely proportional to the magnification M.

Finally, the effect of the mounting medium arises from the same cause as the greater N.A. possible in dense media —*i.e.* from the refraction of cones of rays when entering the dense medium. Thus, if the objective OO in Fig. 83B had its focus in air at F, the introduction of a denser medium at MM would lengthen the cone so as to have its apex at F′.[1] The entering surface MM being flat (*i.e.* the underside of the cover-glass), the reasoning applied in determining the value of a given angle in a dense medium as compared with the value of the same angle in air, applies with the result that the cone to F′ is very approximately n times (n being the refractive index of the medium) as high as that corresponding to air which has its apex at F. The distances from F and F′ respectively at which these cones attain a certain diameter (= a given diffusion) are evidently in the same proportion; consequently the depth of focus is proportional to the refractive index of the mounting medium.

[1] See Addenda for a further explanation.

From what has been said we may now state that the depth of focus can be expressed by the formula—

$$D = \text{a Constant} \times \frac{n}{M \times N.A.}$$

The Constant depends on the diffusion allowed. If this be assumed to be the usual quantity $\frac{1}{100}$ in., then with the aid of our first figure we reason thus: Let the N.A. (see page 82) be ·1 or $\frac{1}{10}$; then the diameter of the object-glass will be one-fifth its focal length;[1] in other words, the cone, having its apex at F, will converge at the rate of 1 in 5. It will, therefore, attain a diameter of $\frac{1}{100}$ in. at a distance of $\frac{5}{100}$ from F, but on either side of F; hence the total range within which the cone is below $\frac{1}{100}$ in. in diameter will be $\frac{1}{10}$ in. This, therefore, is the depth of focus with an objective of N.A. $\frac{1}{10}$ on an object in air ($n = 1$) provided there is no magnification (as otherwise we should have had to allow less diffusion in the object in order to keep within our limit in the image), which mathematically means M = 1. Introducing into our formula $D = \frac{1}{10}$, $n = 1$, M = 1 and N.A. = $\frac{1}{10}$, we obtain—

$$\frac{1}{10} = \text{Constant} \frac{1}{1 \times \frac{1}{10}},$$

whence we obtain the value of our constant—

$$\text{Constant} = \tfrac{1}{10} \times \tfrac{1}{10} = \tfrac{1}{100},$$

which gives us the depth of focus in inches when a diffusion of $\frac{1}{100}$ of an inch is allowed in the image, as—

$$D = \frac{n}{100 \times N.A. \times M}.$$

So far we have assumed the point F in sharp focus to be absolutely fixed, which implies that the image is formed at a fixed distance from the eyepiece, as, for instance, on a photographic plate or on the retina of an eye devoid of accommodation. When the observer uses his accommodation a further visual term must be added to the formula. As accommodation stands for the ability of seeing sharply at various distances, it therefore

[1] Because the N.A. of a single lens may be said to be the ratio of *half* its diameter to its focal length; so the *full* diameter in this case becomes twice $\frac{1}{10}$, equalling $\frac{1}{5}$ the focal length, or a ratio of 1 in 5.

involves the formation of the final visual image at various distances below the eyepoint, which in turn implies shifting of the conjugate points in the object. By differentiating the usual magnification formula $\left(\frac{I}{l} = \frac{I}{F} - \frac{I}{L}\right)$[1] it is found that the magnification in depth is the square of the magnification in diameter;[2] hence the visual term D_v in the depth of focus

[1] Where L = long conjugate, F = focal length, and l = short conjugate.

[2] With reference to magnification in depth, being the square of the magnification in diameter, the following, which is from the pen of Prof. Conrady, will be welcome as a mathematical proof.

The figure (Fig. 83c) by the dotted line shows that $\frac{L}{l}$ is the magnification in the usual sense; by similar triangles, to find the magnification in depth,

Fig. 83c.—The magnification in depth equal to the square of the magnification in diameter.

we apply the formula to a point more distant from the lens by dl, which will have its image at a distance less than L by a quantity dL to be determined. Our formula gives—

$$\frac{I}{l + dl} = \frac{I}{F} - \frac{I}{L - dL}.$$

Subtracting this from the original formula, and multiplying each term with a quantity equal to unity, brings the two sides of the equation to a common denominator, which after transposing becomes—

$$\frac{dL}{dl} = \frac{L^2 - L \times dL}{l^2 - l \times dl}.$$

This is a rigorous but inconvenient formula. To obtain the differential, we imagine dl—and consequently dL—to become smaller and smaller; the result is that the term L × dL becomes more and more insignificant, besides L^2 and "in the limit," when dl has become infinitesimally small, vanishes. Then we get the differential coefficient—

$$\frac{dL}{dl} = \frac{L^2}{l^2} = \left(\frac{L}{l}\right)^2,$$

$\frac{dL}{dl}$ is the desired magnification in depth. $\frac{L}{l}$ is the usual linear magnification, and the equation proves that the first is the square of the second.

formula must be inversely proportional to the square of the magnification, or—

$$D_v = \frac{\text{a constant}}{M^2}.$$

One concrete example will enable us to determine this constant, provided we assume a definite range of accommodation. Let us take it then that the normal range is from 10 in. to infinity. Assume a magnification of 100; this would mean an equivalent focal length of the entire microscope of one-tenth of an inch; a lens of $\frac{1}{10}$-in. focal length produces the image of an infinitely distant object at its principal focus, that of an object at 10 in. distance at one-hundredth of its focal length, or one-thousandth of an inch from the principal focus. With magnification × 100 the accommodation depth of focus is therefore $= \frac{1}{1000}$ in. Introducing as above we again determine the constant, viz.: $D_v = \frac{1}{1000}$ and $M = 100$, which introduced into the formula for D_v gives—

$$\frac{1}{1000} = \frac{\text{constant}}{10000} \therefore \text{Constant} = 10.$$

Hence, for accommodation 10 in. to infinity, $D_v = \frac{10}{M^2}$.

Visually we therefore find the depth of focus—

$$D + D_v = \frac{n}{100 \times \text{N.A.} \times M} + \frac{10}{M^2}.$$

Thus for N.A. ·3, × 100 (M), mounting medium $n = 1{\cdot}5$, $D + D_v = \frac{1}{667}$ in.

Here note, two-thirds of the total depth is due to eye-accommodation, and so is lost in photomicrography, particularly noticeable in low-power work, although negligible with high magnifications.

If we introduce $n = 1{\cdot}5$, N.A. 1·4, and $M = 1000$, such as obtains when using a $\frac{1}{12}$-in. objective upon an object mounted in balsam and a magnification of 1000 diameters, we find the depth of focus is only $\frac{1}{48275}$ of an inch!

CHAPTER VI

EYEPIECES

EYEPIECES, or oculars, as they are often called, are really of several kinds, but only two—the Huyghenian and Ramsden—will here be mentioned, as special forms are explained in the chapter on the auxiliary apparatus for the microscope.

The Huyghenian may be of two varieties, the *Ordinary* and the *Compensating*. See Addenda for a new type by Swift & Son.

The **Ordinary**, which is the form more commonly met with, may be readily distinguished from the Ramsden by the fact that it is composed of two single lenses (the one nearer the eye being called the *eye*-lens, whilst the other is termed the *field*-lens), the convexity of each being turned *towards* the object in use on the microscope. The invention of this combination is usually ascribed to Huyghens, the celebrated astronomer whose name it bears, but there seems good ground for believing that Campani also devised an ocular much of the same type. From what can be gathered from the literature of the subject, it would seem the actual invention was of the nature of a happy inspiration rather than the result of any strict philosophical inquiry, but anyhow, be that as it may, the eyepiece, in some form or other, seems to be one of the best, both for the telescope as well as the microscope.

In the *high*-power combination for the microscope, the condition of achromatism is best fulfilled when the field-lens has a focal length three times that of the eye-lens, the two being separated by a distance (d) equal to half the sum of their focal lengths—

$$d = \frac{f + f_1}{2},$$

but for *low*-power magnification there is usually a change of both these focal lengths, 1 to 1·5 being found about the right proportion, whereas for medium power about 1 to 2.

The passage of the rays through the lenses should now be explained by consulting Fig. 84, where O is the objective, F the field-lens, and E the eye-lens. Dealing with those rays which emanate from a point about the arrow-head, and presuming for the moment they are monochromatic, they would focus at A if left undisturbed, but the interposition of the field-lens F refracts them to A′, where the image is formed. Seeing, however, that this image plane is in the focus of the eye-lens, once again the rays are refracted, being bent towards the axis at P, where the eye is placed. But they are at the same time rendered parallel, which is the condition best suited for the eye to see with. It should be mentioned here that a mistake

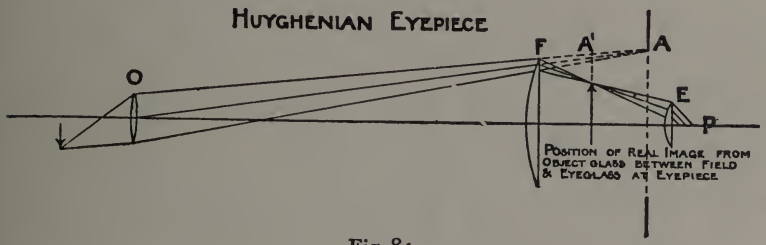


Fig. 84.

is often made concerning the expression "that the rays leave the lens E parallel." It is not meant that they issue parallel *to the axis*, as often erroneously understood, but that they issue parallel with *one another*. This is obvious with a little consideration, for, if they were parallel with the axis, the eye could never get the entire beam into it through the pupil, and incomplete vision would inevitably result, as will hereafter be evident.[1] This then is the path of the rays for a monochromatic beam; but light from an object is not monochromatic in the ordinary way, hence we must now show how the eyepiece causes such perfect achromatism. In Fig. 85, upper part—which is upon a necessarily much larger scale than the preceding diagram—the passage of the red and blue rays is alone shown, the condition depicted being exaggerated very extensively but serving to illustrate the point at issue. The incoming beams if left alone would focus at A as before, but meeting with the field-lens F are refracted to focus again at A′. If there

[1] How to ascertain the diameter of this pencil, see page 114.

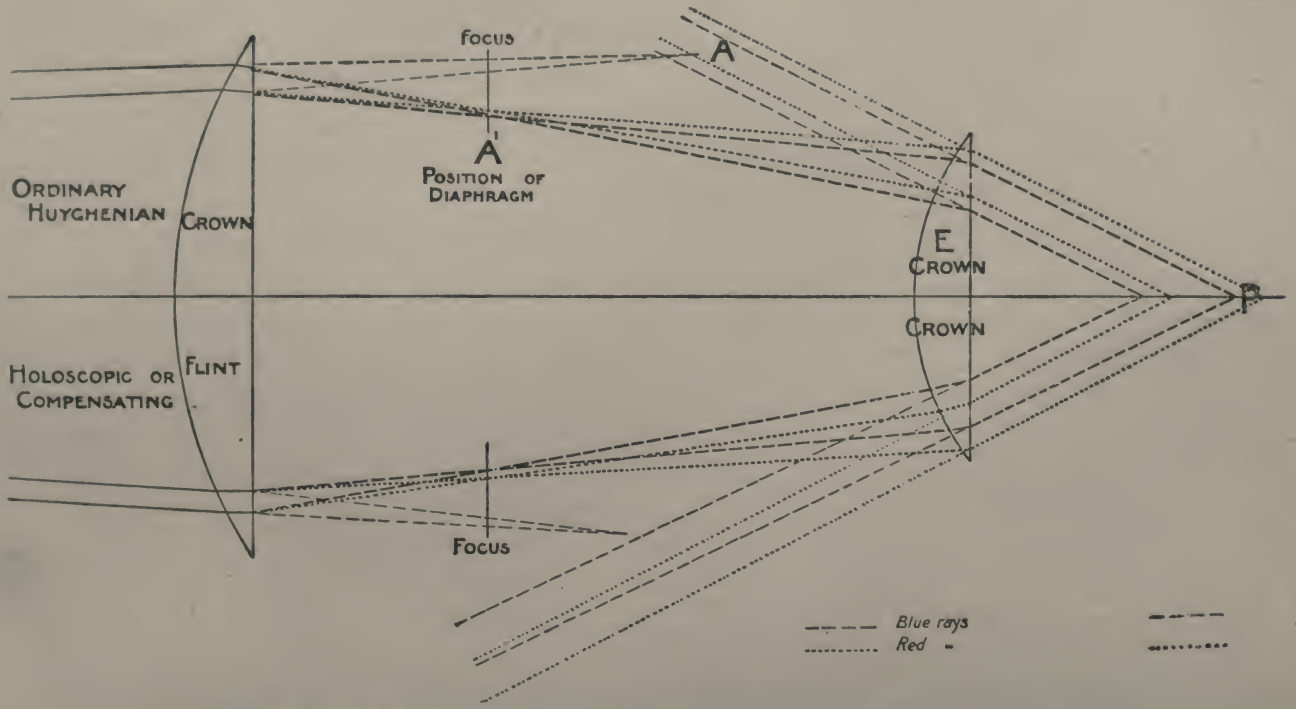


Fig. 85.—Passage of rays through ordinary Huyghenian, Holoscopic, and Compensating eyepieces.

were no eye-lens, *and F were used by the eye as a single lens*, it would produce a *virtual* image with colour fringes in the outer part of the field, for owing to the dispersive power of the glass the red rays sent out by the object would be bent to a less extent than the blue ones, and each point of the virtual image would be drawn into a spectrum with the red end towards the optical axis (see Fig. 85, lower part). But in the Huyghenian combination, where an eye-lens *is* used, the rays—shown in Fig. 85, upper part—cross and become still further separated as they fall upon E. It is seen now that the blue ray meets the eye-lens sensibly nearer the optical axis than the red one. As any lens may be regarded as a prism with a refracting angle increasing from the optical axis, it further becomes apparent with but little consideration that the red ray meets a prism of a greater refracting angle than its companion of blue, and is for this reason bent *more* than its fellow. It is true some of this advantage is counteracted by the difference of the refractive indices for the two colours (about as 1·51 is to 1·52), but with a sufficient separation between eye- and field-lens the first effect greatly predominates, and to such an extent as to cause the red and blue rays to emerge from the eye-lens to P sensibly parallel one with another. *It is this that produces the achromatic image.* A diaphragm is placed at the focus of the eye-lens.

The **Compensating** Huyghenian ocular is only an extension of ideas—the wish of the computer being in this instance to further accentuate the condition of things to such an extent that by a crossing of these rays in the formation of the virtual image as seen by the eye, the red image shall be made *larger* than its blue companion, and to just the right extent to compensate the opposite error of the apochromatic or other high-power objective.

This is effected by using denser glass and greater separation (as in the holoscopic ocular of Watson or a similar one formerly made, but now discarded, by Swift); or by substituting an over-corrected *achromatic* lens for the simple eye-lens. On looking at the lower half of Fig. 85 this is well seen, although again in exaggeration. It shows the condition that obtains with the holoscopic eyepiece. The rays of the two colours are seen well separated now as they arrive at the crown lens

by a sensibly greater interval than in the former simple achromatic ocular. This increase of separation causes the red rays to issue forth to form the virtual image—if we may so speak—as seen by the eye, *not* parallel with the blue, but diverging and crossing in such a manner that the red virtual image becomes larger than that formed by the companion colour, for the purpose which we have already stated. The same explanation practically holds good if the compensation is effected by over-correcting the compound eye-lens ; and it is this state of things that gives rise to the red edge of the diaphragm—placed in the focus of the eye-lens—which furnishes a ready method for rapidly distinguishing in Huyghenian oculars (and usually in the Ramsden too) the compensating from the ordinary. From what has been said, it is evident that as the Huyghenian eyepiece has its focus inside between the lenses, it cannot be used as a hand-magnifier like the Ramsden,[1] or for micrometer work where the threads have to be focussed simultaneously with the object.[2]

The **Ramsden Ocular** may be at once distinguished from the former type of eyepiece as it consists of two lenses, the convex surfaces being turned towards each other. The general path

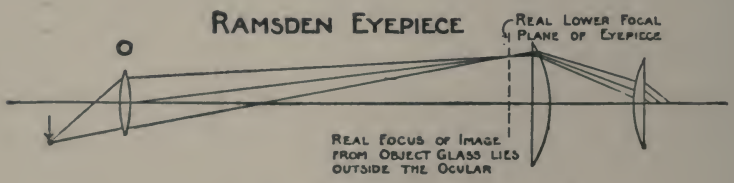


Fig. 86.

of monochromatic rays is shown in Fig. 86. The best form theoretically is where $f = f' = d$, that is to say where the focal length of each lens is equal, and the distance apart equal to the focal length of either. But as this condition would throw

[1] It should be noted that as the selection of the focal lengths is somewhat different in the case of the Huyghenian ocular *for the telescope*, that such combination if turned upside-down can be used for a somewhat indifferent hand-magnifier.

[2] This is the case, ordinarily speaking, but if the field-lens in the Huyghenian be entirely removed, and the eye-lens made to focus the threads, it can then be used.

the lower focal plane of the eye-lens into the field-lens and the upper focal plane of the field-lens into the substance of the eye-lens, the arrangement cannot be strictly fulfilled. To avoid this, which would cause all imperfections in the glass of the field-lens, or any dust upon it to be annoyingly visible to the eye when the ocular was in use, the two focal lengths are usually made slightly different and the separation less than that theoretically demanded. The consequence of this arrangement is to deprive the eyepiece of its freedom of colour, for chromatic *under*-corrections (taken in the sense of a single lens) become painfully apparent, whilst the focal planes still remain inconveniently close to the lenses. These drawbacks can only be overcome by the use of *achromatic* combinations of crown and flint glass; hence, although the lenses in the best form of Ramsden eyepieces *look* the same as in the cheaper type, they are really different, for in point of fact each lens is compound, the components being cemented together. Cheap Ramsden eyepieces may therefore be regarded with suspicion.

In the *compensating form* the same idea of manufacture is carried out, but to a higher order, by introducing a sufficiently strong concave flint element to produce a chromatically over-corrected combination. The Zeiss form of arrangement is shown in Fig. 87. Here again the whole appearance of the rays is greatly exaggerated, but it suffices to show how the extremely dense flint glass separates the blue and red rays so much that in the virtual image the red component is larger than the blue which is required to correct the opposite condition in the objective. On the other hand the figure serves to show that if the flint were not so dense a combination of this particular form might be computed where the virtual image would be purely achromatic, for the ocular to be used as an ordinary Ramsden eyepiece. As a matter of fact, with the compensating eyepieces manufactured by Zeiss the Huyghenian type is in being for those up to the nominal × 6, after which the Ramsden form takes their place. This selection is found to be the more convenient because of arranging a suitably distanced eye-point and for enabling the adjustment to be such that the lower focal planes of the entire series shall lie in the same position when the oculars are inserted into the draw-tube; a change of eyepieces therefore necessitates scarcely any change of

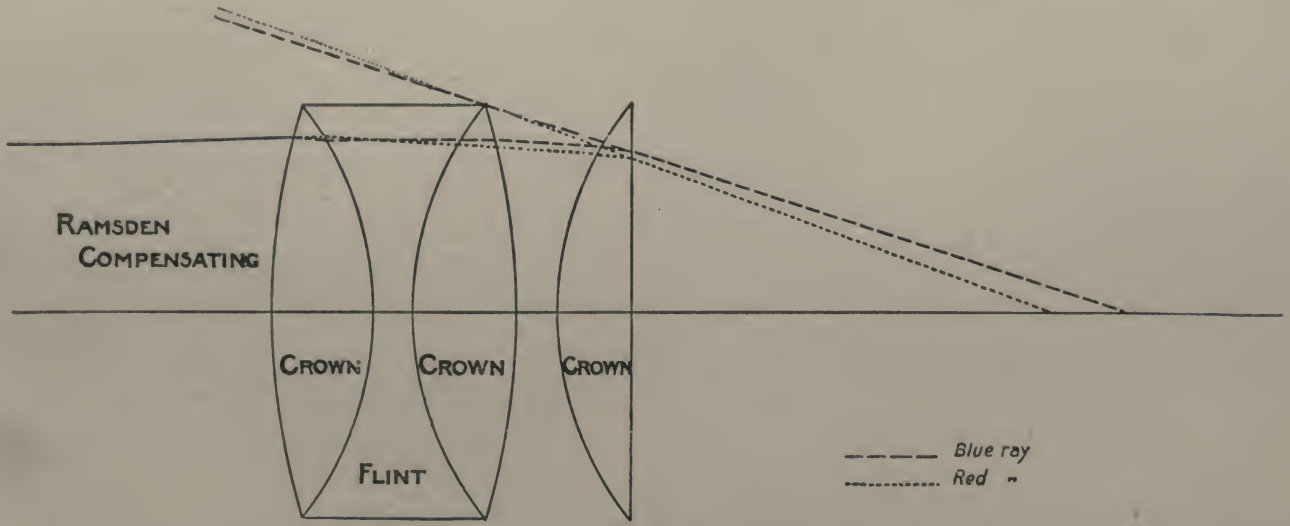


Fig. 87.

focus simply from the fact that the distance between the upper focal plane of the objective and the lower focal plane of the ocular remains constant. An ocular constructed on this principle is called par-focal, but to this we shall refer later on.

In the holoscopic eyepiece the over-correction for the compensating effect is simply produced by separating the lenses —one of which is flint—sufficiently to make the red image larger than the blue. When these eye-pieces were first introduced, the high powers had an extremely near eye-point, and, being unpleasant to use on this account, we drew attention to the defect. In the new series, however, this trouble has been greatly removed. Hence, besides their use for testing lenses, they are now employed by several for ordinary work, as when the components are pushed *together* they are serviceable for achromats, and when *separated* can be employed for apochromats. Care is required in using them, however, for without careful adjustment the performance of an otherwise good objective may be seriously impaired.

As with all classes of Ramsden eyepieces the focus is without the ocular, so they can all be used as hand-magnifiers, and in consequence are mostly employed for micrometers where measuring-threads are employed; the webs being first set in the focus of the eyepiece, the *eyepiece as a whole—i.e.* webs and all—being moved nearer to or farther from the object when focussing it. By this means the webs and the object are simultaneously in adjustment at one and the same time and the different dimensions of the object can be taken. To this we shall refer again when speaking of measuring objects, when we also point out how the Huyghenian form of ocular, simple or compensated, can be employed for much the same purpose, although in a different manner.

In the usual form of Huyghenian oculars a change of focus is necessary with each power, but Messrs. Bausch & Lomb and The Spencer Lens Co. have devised and made a series all *par-focal* in which these eyepieces, like the compensating, have their lower focal planes in a constant position so that *no* change of focus (beyond the merest trifle) is necessary.

Oculars are made to several gauges each requiring the draw-tube of the microscope to be made in accordance. The following

sizes are those mostly in use, being in accordance with a resolution of the Council of the Royal Microscopical Society, December 1889:

Size 1, 23·2 mm., to fit draw-tube ·9173 inch, or 23·300 mm.
(„ 2, 26·3 „ „ „ 1·04 „ 26·416 „)
„ 3, 32·2 „ „ „ 1·27 „ 32·258 „
(„ 4, 35·8 „ „ „ 1·41 „ 35·814 „)
Sizes 2 and 4 are dropping out of use.

Of these the first is the usual Continental diameter; the second is the mean diameter for the English or long-tube microscope; the third, the mean for medium-sized binoculars; whilst the last is that of the largest-sized binoculars. There are other odd sizes.

Compensating oculars, both of Huyghenian and Ramsden, may

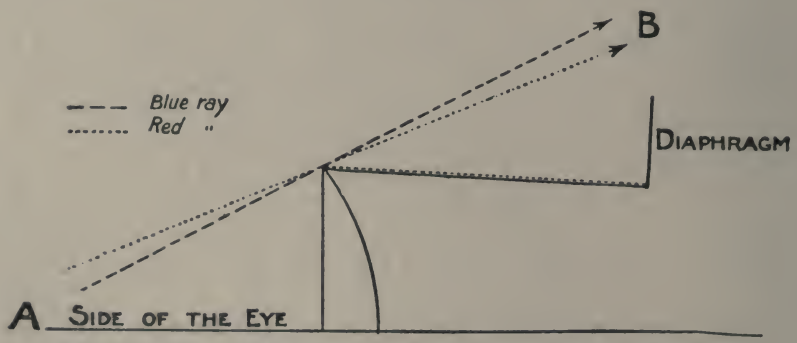


Fig. 88.—Ordinary *Unachromatic* Eye-lens. Blue ray outside, hence blue margin to field.

usually be distinguished from *ordinary* eyepieces by looking through them when held up to the light: the former showing a yellowish red margin to the diaphragm, whereas the latter produces a blue fringe to the field. It has been often asked how the difference in colour is to be explained, and the writer is not able to point to any text-book wherein any scientific information upon this point is to be found; hence the few following remarks may be of interest. Speaking of the Huyghenian form of either compensating or ordinary ocular, the first point to bear in mind is that the coloured rays in question are not due to the eyepiece *taken as a whole;* by which is meant they are not due to a conjoint action of the two component lenses, the field and the eye-lens, but to the action of

the latter *entirely*. Indeed the former may be considered merely as a condenser, for it may be removed and the effect is unaltered!

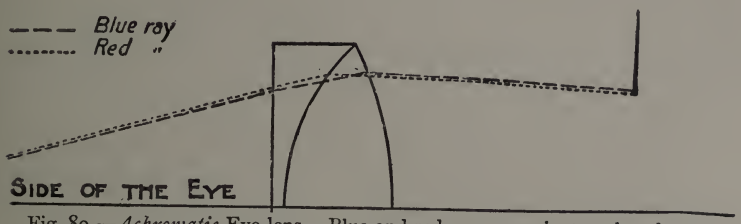


Fig. 89.—*Achromatic* Eye-lens. Blue and red rays remain together, hence colourless margin to field.

If Fig. 88 be consulted, the red and blue rays (only two colours are taken) from the edge of the diaphragm to the lens will be seen split up and separated at A. But the eye *sees* them as if in the direction of B, where the blue ray lies outside the red—

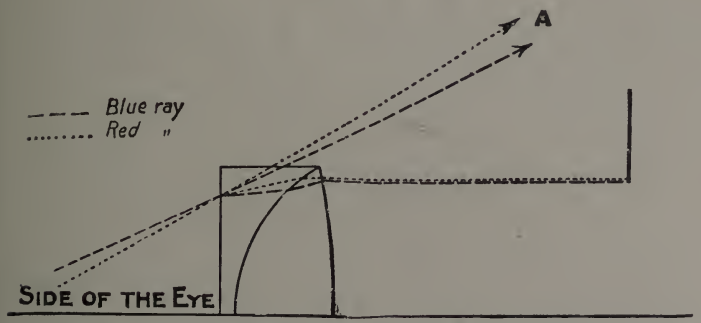


Fig. 90.—*Over-corrected* Eye-lens. Red outside, blue inside, hence red (or rather, owing to its greater brilliancy, yellow) margin to field.

hence the blue margin to the field. In Fig. 89, which represents the case of the *achromatic* eye-lens, the blue and red keep together, and so a colourless margin would be furnished to the edge of the diaphragm. But in Fig. 90, of the *over*-corrected eye-lens (as in all Zeiss compensating Huyghenian oculars), the red is bent so much more than the blue that in the virtual image, as seen by the eye, it lies outside at the arrow-points A, thus explaining the reddish yellow fringe to the field.

Curiously these distinguishing characteristics of colour-rendering do not obtain with the holoscopic ocular when adjusted to become either an ordinary or a compensating ocular. This is explained by consulting the lower part of Fig. 85, when it is observable how the rays do not take up their final position in the virtual image until at least some *sensible distance from the eye*; hence a blue margin is seen in either case, that is to say, when the eyepiece is adjusted for the ordinary or compensating effects.

The cause of the red edge to the diaphragm in the case of the Ramsden compensating ocular needs no further comment, as the matter explains itself by the study of the previous figures.

Before leaving the subject of eyepieces, seeing the importance that the emerging beam should always be the required diameter to enter and pass the pupil of the eye, it would be an omission not to show how to ascertain such diameter and to point out the philosophy by which such computation is arrived at, for little reference is made to the subject in most handbooks on the microscope. See Addenda for different foci of oculars.

Of course the normal diameter of the pupil varies with different individuals, but it should also be borne in mind that any pupil varies from its normal diameter at different times according to the direction and the intensity of the light impinging on the eye. To fix the actual diameter of the pupil, or its variation from the normal under different circumstances, has been a desideratum under consideration by many physiologists and others. Taking a mass of evidence it may be presumed that, under the circumstances which hold with the microscopist, the usual working diameter is not far from one-tenth of an inch; but whilst stating this somewhat empirically it must be understood with the formulæ that are given and the discussion that follows, any diameter can be interpolated. Before actually commencing to point out how to ascertain the diameter of the emerging beam from the eyepiece, it should be stated that such diameter is affected by two factors: (1) the magnification of the eyepiece, and (2) the N.A. of the objective. (1) In Fig. 91 let OL be a lens of the same focal length as the objective and EL another simple lens of the same equivalent focal length as the ocular. These primitive forms are taken for simplicity's sake

and in no way affect the general reasoning. Rays from the objective OL focus at F_1 and oblique pencils at F_2, all other rays being excluded for purposes of description. Half the inverted arrow A would be formed at this situation. The rays at F_1, the

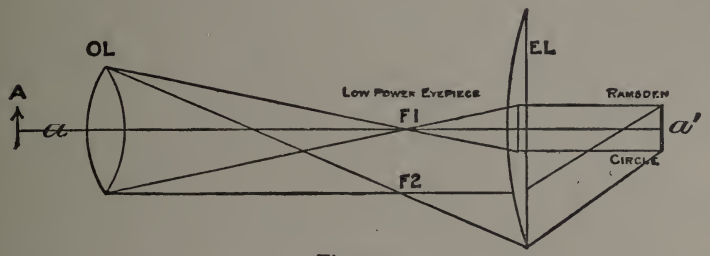


Fig. 91.

focus, pass on to the eye-lens EL and issue parallel, one on one side and one on the other of the axis *aa'*. Those focussing at F_2 pass on to the periphery of EL, are rendered parallel one with the other, and are bent towards the axis *aa'*, meeting those from F_1 as the diagram shows. This point of union is called the Ramsden circle. In Fig. 92 the same argument holds, but as

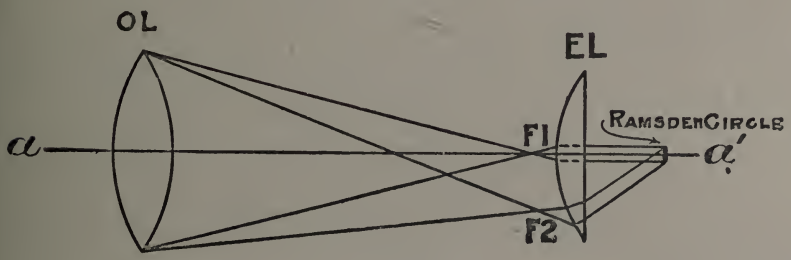


Fig. 92.

the eye-lens is of shorter focal length (a higher magnifying power) a smaller Ramsden circle is formed. It is obvious then that the higher magnification, with its smaller Ramsden circle, needs a smaller pupil than the larger circle shown in Fig. 91, where the magnification was less. Hence the greater the magnification the smaller may the pupil be.

(2) In Fig. 93 let OL be an objective and EL the *eye*piece lens as before. Let the rays AA be alone considered. This repre-

sents a lens of low numerical aperture, and the beam to enter the pupil is small as indicated by A′A′. Now let the full aperture BB be considered—a lens of larger numerical aperture;

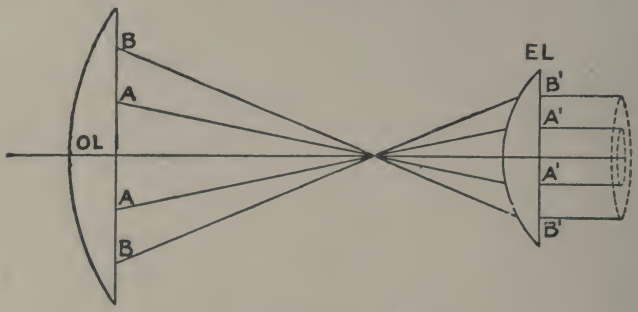


Fig. 93.

the corresponding pupil B′B′ is larger: hence the *higher* the N.A. of the objective the *larger* the pupil required.

As, however, the N.A. remains constant with a given objective whilst the magnification is raised or lowered, so it is only with the first statement we have principally to deal. This, for convenience of description and to clear the mind so as to understand better what follows, may be put another way. The diameter of the issuing beam becomes larger and larger *as the magnification is lessened.* The problem then is to show how to ascertain the *lowest* magnification with a given objective that is permissible

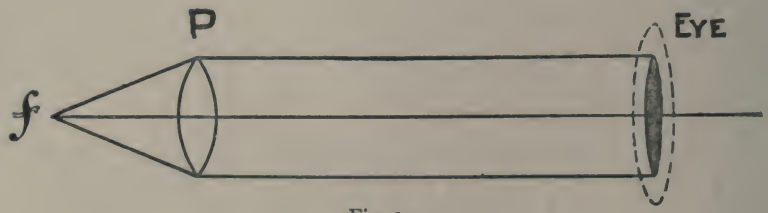


Fig. 94.

to allow the beam from the eyepiece completely to enter the pupil of the eye, say of $\frac{1}{10}$ in. diameter. This magnification we state for convenience at once to be equal to 200 *times the N.A.* The argument for proof may be taken as follows:

It must first be borne in mind that the compound microscope,

apart from the inversion of the image, produces the same effect as would be shown by an optically perfect lens of the same N.A. and magnification. In Fig. 94 let f be the focus of the lens which has a clear diameter P.; and in what follows let f^1 be its focal length.

From what has been said in the chapter upon numerical aperture, the general rule will be understood that follows: N.A. $= \frac{\frac{1}{2}\,P}{f^1}$ or half the aperture divided by the focal length.

Transposing this—

(1) - - - - N.A. $= \frac{P}{2f^1}$.

The magnification (at 10 in.) of any lens is $m = \frac{10.}{f^1}$. Transposed this becomes $f^1 = \frac{10}{m}$, m being the magnification under consideration. Introducing this latter into (1) we get—

(*a*) - - N.A. $= \frac{P}{2 \times \frac{10}{m}}$ or N.A. $= \frac{P \times m}{20}$.

In this formula we get the N.A. required to find a pupil of given diameter at a given magnification. Example: What N.A. is necessary to fill, say, $\frac{1}{8}$ in. pupil at a magnification of 200? Then by *a* we find—

$$\text{N.A.} = \frac{\frac{1}{8} \times 200}{20} = 1{\cdot}25,$$

so N.A. 1·25 is required.

If, however, we desire to know the diameter of the emerging pencil with a given N.A. and a given magnification, we have—

(*b*) - - - - $P = \frac{20 \times \text{N.A.}}{m}$.

Example: An objective with N.A. ·65 and a magnification of, say, 650; what is the diameter of the emerging pencil?

$$P = \frac{20 \times {\cdot}65}{650} = \tfrac{1}{50}\text{ in.}$$

Again, on the other hand, presuming a given N.A. and a given diameter of pupil, what is the magnification required so that the pencil may pass the pupil? Then—

(*c*) - - - - $m = \frac{20 \times \text{N.A.}}{P}$

Example: Let the pupil be fixed at $\frac{1}{10}$ in., and the N.A., say, ·65; then—

$$m = \frac{20 \times \cdot 65}{\frac{1}{10}} = \mathbf{130\ diameters.}$$

If now we put this another way, then—

$$m = \frac{20 \times \text{N.A.}}{\frac{1}{10}} = \mathbf{200 \times N.A.,}$$

which shows and furnishes the proof that the assertion made at the commencement of this discussion is correct. This formula also shows that the emerging pencil is only $\frac{1}{500}$ in. in diameter, when the limit of useful magnification (viz. 1000 times the N.A.) is employed.[1]

[1] This subject may be put a different way which appeals better, perhaps, to the direct question, What is the Ramsden circle? The Ramsden circle is really the image formed by the eyepiece, of the full aperture of the objective, measured in the upper focal plane. This image depends on the N.A. and on the focal length of the objective in the well-known manner:

$$\frac{\text{Semi-diameter of the full aperture}}{\text{Equivalent focal length of the objective}} = \text{N.A.}$$

whence we obtain the diameter of the emerging cone of any objective, measured in its upper focal plane—

$$= 2 \times \text{N.A.} \times \text{Focal length of the objective.}$$

The eyepiece forms a *diminished* image of this; the diminution equalling the power of the eyepiece (according to Abbe nomenclature), *i.e.* equal to the number engraved on a compensating ocular. Calling this number M, we have therefore the diameter of the Ramsden circle—

$$= \frac{2 \times \text{N.A.} \times \text{Focal length of objective}}{\text{M}}. \quad - \quad - \quad - \quad - \quad (1)$$

This may be modified by introducing the initial magnification of the objective $= \frac{250 \text{ mm.}}{\text{focal length}}$; whence we obtain by transposing—

$$\text{Focal length of objective} = \frac{250 \text{ mm.}}{\text{Initial magnification}};$$

and introducing this in (1) we get—

$$\text{Diameter of Ramsden circle} = \frac{500 \text{ mm.} \times \text{N.A.}}{\text{Initial magnification} \times \text{Eyepiece number}}.$$

The denominator is, obviously, the total magnification; hence the simple rule—

$$\textbf{The diameter of the Ramsden circle} = \frac{500 \text{ mm.} \times \text{N.A.}}{\text{Total magnification}}.$$

The *position* of the Ramsden circle (or eye-ring, as it is sometimes called) is where the eyepiece forms the image of the aperture of the objective, slightly above the upper focal plane of eyepiece. Its distance from the *top* lens depends entirely on the construction of the ocular, and cannot be foretold anyhow without a somewhat detailed computation.

CHAPTER VII

MAGNIFICATION

The Evaluation of Objectives and Oculars and the Limits of Useful Magnification

To know how much an object is magnified is always a desideratum with the microscopist, but the importance of very great exactitude is often over-estimated by the student, who in addition is apt to think that amplification, taken as such, is everything, and who quite often forgets that unless it is accompanied by sufficient "aperture" of the objective—which means by a sufficient inherent power of resolution—is useless and unprofitable. Concerning this important detail, we may mention the subject has been dealt with in the section devoted to the explanation of Numerical Aperture (Chapter V.), and that it is referred to again later on.

Before explaining the methods that can be adopted for the evaluation of objectives and oculars, however, a few words should be said in defining the terminology used, which in recent years has undergone a certain amount of alteration. With the older microscopists two terms were in familiar use. An object was said to be magnified so many "*times*" (or "areas"), or so many *diameters*.

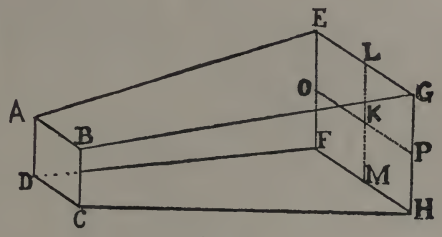


Fig. 95.

Let Fig. 95 be considered. An enlarged image of the object ABCD, we will say, is cast upon a screen at a given distance, and shown as EFGH. If now a pair of compasses be taken, it will be found that EG is twice AB, GH twice BC, FH twice DC,

and EF twice AD. It is not difficult to understand the dotted lines; LKM and OKP are drawn equally, dividing EG and FH and EF and GH respectively. Then EL and LG are each equal to AB; GP and PH each equal BC, and so on with the other sides. A little more attention and it is evident that there are four squares, each equalling ABCD; so this object is said to be magnified four "times" or four "areas." But it is equally obvious that ABCD is only enlarged twice in each direction—twice from above downwards, and twice from side to side. Hence, when speaking in what is called linear measure, the object is said to be amplified *two* "*diameters*." This holds good for any magnification, and in Fig. 96 the little square *a* is magnified *five* diameters in the enlarged picture by its side; but as the larger diagram has twenty-five little squares in it, so it can be said to be twenty-five "times" as large in area.

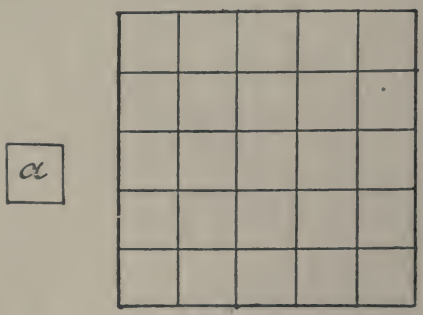


Fig. 96.

This is a simple explanation of the two different terms that used to be in vogue. But the scientist never spoke in terms of areas at all, for his magnifications were always expressed in diameters; hence, as time passed along, this expression of "times" simply died out of use. In comparatively recent years, however, it has become the fashion in daily use to speak of the magnifying powers of oculars in a manner that has led to a little confusion in the minds of some which we had better at once set straight. We refer to the expression that an eyepiece magnifies "times so much"; for example, we read "that a times eight ocular (written × 8) was used in conjunction with a quarter-inch objective." Now what is universally understood in the present day is that the eyepiece in question had an initial magnifying power of eight *diameters*; but the mistake that has been made in the minds of some was, that "times eight" meant not *diameters*, but *areas* instead. Of course this has arisen simply from the unfortunate meaning which used to be attached to the word "times" as

synonymous with *areas*, whereas now it is meant to be the same as *diameters*.[1]

Previous to the introduction by Professor Abbe of his system of apochromatics, he very rigorously reviewed the methods in vogue at the time for evaluating objectives and oculars. The difficulties and inconveniences in obtaining the real magnification of an object at the eye end of the microscope, which were thus made apparent to this careful observer, led him to reconsider the whole subject from quite a different standpoint, with the result that he originated and gave to the scientific and manufacturing world what has been called his "new idea" upon evaluation ; and his method, which requires some little attention to follow, has been slowly but surely adopted by all, or nearly all, the leading opticians both in England and on the Continent.

Before proceeding to explain "the Abbe evaluation method," however, we must first show the older methods which obtained up to the time in question, and which even now are employed by microscopists when dealing with objectives and oculars not arranged upon his special system of nomenclature.

To obtain the magnification of an object as seen at the eye end of the microscope in the easiest way, is by the use of what has been called the "rational system." It is based upon direct experiment, which, although tedious to carry out, is easily made by any intelligent observer in the following manner :

Place any objective—say an inch—on the microscope, and use first what is called an "A" or No. 1 eyepiece. Having procured a stage micrometer (which is only the name for a cover-glass ruled with lines of some definite distance apart and fixed on an ordinary 3 × 1 slip),[2] and having focussed any two lines which are separated by an interval thought to be of convenient dimensions, a piece of ground glass is placed at a distance of 10 in. from the eyepoint of the ocular—which will be found 5 to 10 mm. above its eye-lens. A pair of compasses is now used to measure the interval of the lines as seen on the ground glass, which interval (having been converted into terms of the

[1] This short explanation is interpolated here because the original distinction between "number of times" and "diameters" dies hard in the minds of certain microscopists of the old school.

[2] Micrometers, or graticules, as they are now called, made by Rheinberg & Co., 51-60, Holborn Viaduct, by a new process, are certainly the finest we have ever seen. See Addenda.

same order as those on the micrometer—that is to say, "so much of an inch, or so many millimetres") is then divided by the distance known to exist between those on the stage micrometer. The quotient thus obtained is the linear magnification required very approximately.[1] This process must be repeated for every eyepiece and with each objective in succession, and a table of reference made. Although this constitutes, perhaps, the simplest method of obtaining the existing magnification afforded by a given combination of objective and ocular, still it is obvious that fresh measurements must be made with every change in length of draw-tube. The consequence of this is, that when an observer draws out his tube to correct the aberration of an extra thin cover-glass, all his magnification values are immediately upset, and he has again to recalculate them for the special "draw" in question if he desires any approach to accuracy. To meet this difficulty, opticians frequently not only give a table of magnifications for the combination of certain of their own objectives with their different oculars, but also add an appendix to show how much increase of amplification is caused by additional lengths of the draw-tube. For approximations this tabular system amply suffices, but it is not sufficiently accurate to please some minds.

Practically and theoretically it is really much better for the microscopist to ascertain, once and for all, the actual values for each objective and eyepiece *separately*, so that magnification at normal tube-lengths is immediately found by multiplying together these two factors, any increase in the tube-length proportionately increasing the magnification of the objective. By this is meant, that if such value at a normal tube-length of any given *objective* be, say, 60, and the tube has been additionally drawn out one-sixth the entire distance from objective to eyepiece, its initial value is increased thereby *approximately* to the same extent: we say approximately because hereafter a slight modification, it will be shown, is necessary if great accuracy be required. Having ascertained this new quantity, the amount

[1] Suppose the lines were known to be ruled $\frac{1}{100}$ in. apart in the micrometer, and they appeared on the ground glass to be separated by $\frac{1}{10}$ in. instead, the magnification would be ten-written X 10. See Index for Mr. M. D. Ewell's results (past President American Microscopical Soc.) of testing various stage micrometers not including the new ones by Rheinberg. See Addenda.

has only to be multiplied by the magnifying power of the eye-piece in the usual manner to complete the operation.

How to ascertain these separate values, however, involves some little thought and explanation, and seeing that such very fittingly forms an introduction to and explains the reason why Professor Abbe introduced his elegant and original modification of the entire subject from beginning to end, we will at once proceed to put the reader in possession of the whole matter from its very commencement.

Theoretically, the process of obtaining the power of an objective consists in measuring the amount of amplification of the object in that particular plane within the draw-tube where the computer has elected his focussed image shall always lie. But it is here that difficulties at once arise. If the optician were asked to furnish the position of the "image plane," as it is sometimes called, he would not describe its situation as being at such and such distance up or down the draw-tube, which *without certain qualifications and explanations* might be cumbersome and unscientific, but as so many millimetres distant above the upper focal plane of this objective (see Appendix). But where is this upper focal plane of an objective to be found? How can it be located? And what is the distance above it that is usually selected for the image plane to lie in? None of these details are ever given with objectives, even though manufactured by the best of opticians, and we cannot recollect having met with an English text-book that even mentions the question. When Professor Abbe took the matter into his consideration all these positions were in a chaotic state of confusion, for one computer chose one set of data whilst a second chose another. The consequence of this was that much had to be presumed, and the secondary consequence was that many objectives were thought to magnify more or less than others, although of the same focal length! To this confusion was added yet another, which arose from the fact that opticians insisted on calling their oculars by either letters of the alphabet A, B, C, D, or by numerals 1, 2, 3, 4, without giving any hint whatever of their relative magnifications. It is usually now adopted by convention—owing largely to the masterful influence of Professor Abbe's writings and teachings—that the distance of the image plane of focus from the upper focal plane of the objective, called "the

optical tube-length," shall be 180 mm. for the short or Continental form of microscope, and 270 mm. for the long or English form. But no such definite position has been decided, we believe, for the position of the upper focal plane of the objective.[1] It will be shown hereafter that that is *one* of Professor Abbe's "proposals," and that such proposal is getting more and more accepted by all English and Continental manufacturers. Seeing that the real magnification of any objective can be at once calculated by dividing its *optical* tube-length by its real focal length, without the trouble of actually measuring the amplification on a ground glass or other screen at the image plane of focus, so it is evident that it is of more than academic interest for the student to be able accurately to determine and locate the exact situation of this plane with any given objective under examination.

From what has been said, the practical microscopist will readily admit that the magnification at the image plane will largely depend upon the length of this optical tube; but it may not be immediately apparent how it is also largely affected by the focal length of the combination. This will be better understood by the following explanation dealing with, say, a 2-mm. objective. If it were possible to place a piece of ground glass at its upper focal plane, it would be found that there was no magnification at all. If the screen were removed 4 mm. from the same starting point, then the amplification would be found to be 2; if removed 180 mm., then 90; if 270 mm., 135, and so on. Hence the rule is simply this, that the real magnification of any given objective is always to be found by *dividing the length of the adopted optical tube* (that is to say, the distance of image plane from upper focal plane) by the *focal length*. It is obvious then that the focal length of the objective may be said to be a kind of yard measure—so to speak—of the amplification.

Before proceeding to describe the method for discovering the position of this upper focal plane of any objective, no confusion must be allowed to remain in the reader's mind with respect to the two tube-lengths, of which we shall speak and of which we have spoken. The *mechanical* or *standard* tube-length is the name given to the interval that is included between the shoulder of the objective (when *in situ* on the microscope) and the

[1] It varies, as a matter of actual fact, very sensibly in some cases.

eyepiece end of the draw-tube; such distance being now by common consent fixed at 160 mm. or 170 mm. for the short or Continental instrument, and 250 mm., or 10 in., for the English model. But when the expression *optical tube-length* is employed, it should be understood to mean the distance in millimetres between the upper focal plane of the objective and the image plane where the image of the object would be found in the draw-tube were the eyepiece removed after focussing and adjusting the *mechanical* tube-length to its proper amount. This length, we have just stated, is 180 mm. for the short or Continental instrument, and 270 mm. for the long or English form. It will be seen then that the *optical tube-length is usually 20 mm. more than the mechanical*—at least, that was the suggestion of Professor Abbe—in the case of each type of instrument.

In locating the position of the upper focal plane the first step is to ascertain the focal length of the combination. To do this two measurements of magnification m_0 and m_1 are taken with *two tube-lengths* L_0 and L_1. The distance between the upper focal plane and a micrometer placed in the eyepiece will then differ from the mechanical tube-length by a *constant* quantity x; therefore we should have, theoretically—

$$\frac{L_0 + x}{\text{focal length}} = m_0 \text{ and } \frac{L_1 + x}{\text{focal length}} = m_1.$$

From this we see

$$\frac{L_1 + x - (L_0 + x)}{\text{focal length}} = m_1 - m_0;$$

or—

$$\frac{L_1 + x - L_0 - x}{\text{focal length}} = m_1 - m_0,$$

reducing which we obtain—

(i) - - - $$\text{Focal length} = \frac{L_1 - L_0}{m_1 - m},$$

which being interpreted means that the

$$\text{Focal length} = \frac{\text{difference of tube-lengths employed}}{\text{difference of magnification experimentally found}}.$$

In actual practice to carry this out, two rulings on cover-glasses are obtained, one with divisions ·01 mm. apart mounted on a slip to place on the stage of the microscope, and the other with divisions separated by intervals of ·1 mm. This latter "ruling" should be mounted on a disc of glass of such a diameter that it can be slipped into a *Ramsden* eyepiece, which must be arranged so as to permit the rulings being inde-

pendently focussed *before* the ocular is placed in the microscope. A Huyghenian form may be used for this purpose by *removing the field-lens* and using the eye-lens only, but here, too, arrangements must be made for focussing the rulings before using on the microscope.[1]

The exact distance between the eyepiece micrometer (remembering that the cover-glass ruling in these eyepiece-micrometers is in most cases balsamed *beneath* the disc of supporting glass) and the upper end of the draw-tube must be very accurately ascertained with the eyepiece *in situ*. In an example which follows, this distance was found to be 13·5 mm. Having placed the objective to be tested on the microscope, several measurements must now be made (say four as in the example that follows) and their mean taken, so as to ascertain how many divisions of the eyepiece micrometer equal, say, five divisions of the stage micrometer. Let us say these are as follows :

mm.			mm.			mm.
·05	(say) is found to be contained in	52·8	divisions of the eyepiece micrometer, which equals			5·28
·05	Ditto	53·1	Ditto			5·31
·05	Ditto	52·5	Ditto			5·25
·05	Ditto	52·8	Ditto			5·28
·20					=	21·12

Therefore 1 mm. = 105·6 mm.

The observations repeated on another part of the scale we will say furnish the figures 1 mm. = 104·7 mm., hence the final mean shows that the adopted magnification is 105·15 *diameters*. The draw-tube is now pulled out, say, 60 mm. (making, as it happens, an entire length of 260 mm. from the shoulder of the objective to the end of the draw-tube) and five observations for magnification are again made.

In this case we will suppose—

mm.			mm.			mm.
·04	is found to be contained in	54·0	divisions of the eyepiece of the micrometer, which equals			5·40
·04	Ditto	54·3	Ditto			5·43
·04	Ditto	54·1	Ditto			5·41
·04	Ditto	54·0	Ditto			5·40
·04	Ditto	54·0	Ditto			5·40
·20					=	27·04

[1] If any difficulty arises in the reader's mind concerning this arrangement he is referred to the section on ascertaining the size of given objects, Chapter XII.

Therefore 1 mm. = 135·2 mm. and we will suppose a second series gives 1 mm. as equal to 135·4 mm. Hence the mean shows that the *adopted magnification is* × 135·3. Continuing the precept contained in (i) to obtain the focal length, the difference in the tube-lengths (60 mm.) has to be divided by the difference in the magnification experimentally ascertained; hence—

$$\frac{60}{135{\cdot}3 - 105{\cdot}15} = 1{\cdot}99 \text{ mm.}$$

which is the focal length required.

To ascertain the position of the upper focal plane all that is now required is simply to multiply the magnification obtained either with the tube *in* or with the tube *out* (we will say with the tube *out*) by the focal length experimentally ascertained. Thus 135·3 × 1·99 = 269·2 mm. The position then of the upper focal plane will be found to be 269·2 mm. from the image plane focus, which was, at the position the micrometer rulings had been placed, 13·5 mm. below the upper end of the draw-tube. Adding this amount to the 269·2 mm. makes 282·7 mm., which becomes the *distance of the upper focal plane from the end of the draw-tube when extended in the experiments.* It has been shown that this extension of the microscope from the shoulder of the objective to the end of the draw-tube happened to amount to 260 mm. exactly, so deducting this amount from 282·7 leaves 22·7 mm., *which is the position of the upper focal plane below the shoulder of the objective.* In another set of experiments with another objective this position was found to be 23·3 mm. below the shoulder.

With respect to the evaluation of the *ocular* we must again point out and protest that eyepieces, whether of the Huyghenian or the Ramsden type, are usually numbered with the numerals 1, 2, 3, etc., or by the letters of the alphabet, A, B, C, D lettering that conveys no possible information whatever as to their magnifying power. All that the microscopist knows is that 1, or A, magnifies least and that 4, or D, amplifies most. Some few opticians, it is true, give more information, calling their oculars by their real focal lengths, so that we hear of a 2-in., 1-in., ½-in., and so on. If these focal lengths be accurate we know at once the real magnification by dividing the conventional distance of distinct vision, viz

10 in., by the focal length—hence a 2-in. magnifies five diameters, an inch ten, and so forth. If we are dealing with the simply "lettered" oculars, their magnifying power may be found by using them upon any objective (of which the magnification value has been previously accurately determined) and projecting the image of the stage micrometer on to a screen 10 in. away from the eyepoint (about 5 to 10 mm. above the eye-lens) and measuring how much the final magnification amounts to. Knowing the initial of the objective we divide the final sum as shown on the ground class by the amount, and the quotient gives the magnifying power of the ocular. If for example the magnification power of the objective has been ascertained to be 60, and the final amplification on the screen amounts to 300, we know at once that the eyepiece is responsible for a magnifying power of 5, because 300 divided by 60 = 5.

This method of testing the magnifying power of oculars furnishes true results provided the eyepieces are constructed by the optician who made the objectives; for it is presumed he would have arranged them so that they all fall in the draw-tube the correct distance so that their lower focal planes shall coincide with the image plane of the objective. Unfortunately until Professor Abbe strongly urged a uniform position for all manufacturers to adopt, this was not, and is not even now, always the case; and the consequence of the lack of uniformity is that an eyepiece by one maker may apparently magnify more than that of another (although of the same designation), or that an objective with a certain ocular may appear to magnify more or less than another objective although of the same focal length; obviously because the two manufacturers have not arranged for their oculars to drop in the tube a similar distance. If one were to have dropped too far, the final magnification would of course be too small, because really the optical tube was thus being unconsciously shortened, whereas if the ocular did not drop in far enough, the optical tube being thus lengthened by the error, the magnification would be immediately increased. It is scarcely necessary to add that all manner of confusion was frequently being introduced in this way, and makers were sometimes blamed for sending out an objective which did not apparently magnify enough, or perhaps

one that amplified more than it theoretically should. Once again we stop to point out how these discrepancies urged upon Professor Abbe once and for all to set matters on a distinct footing by introducing a system quite different for his new apochromatic series of objectives and their compensating eyepieces, with which no such fault can be found.

Should the microscopist wish to ascertain the position of the lower focal plane of any eyepiece (see Appendix) he must proceed as follows: Lay across the end of the draw-tube any sort of glass scale with the divisions *downwards*, that is towards the objective, and place on the stage some well-defined object of any kind. Aided by some sort of hand-magnifier let him now focus the object on the stage in such a manner that when he sees the lines of the divisions correctly he sees the object on the stage equally well focussed. This means that the image projected by the objective lies on the same plane as the upper end of the draw-tube upon which the ruled lines have been resting. Having removed the latter rulings, the Huyghenian ocular to be tested is gently slid into the draw-tube and a position found (without disturbing either the focus or the draw-tube) where the eyepiece shows the object on the stage distinctly focussed. The tube of the ocular must now be marked level with the top of the draw-tube, for that is the position of the lower focal plane of the eyepiece in question. If the ocular be a Ramsden, owing to its focus being *outside* the lenses in contradistinction to between them as obtains with the Huyghenian, a piece of tubing must be employed which, while holding the eyepiece at one end, will slip into the draw-tube at the other. After obtaining the position of best focus (as before) a line must be drawn on the auxiliary tube corresponding with the end of the draw-tube. This marks the position of the lower focal plane of the eyepiece in use.

Strictly speaking, all these matters should have been gone into before measurements were made by projecting the image on the ground glass to obtain the final magnification, due regard being paid to such observations: *then* the final amplification on the ground glass divided by the magnifying power of the objective gives the *true* and not approximate magnification of the eyepiece. When these magnifying powers have been obtained for objectives and eyepieces, any of the eyepieces

(tested) can be used upon any of the objectives (tested), and multiplying the values together will give the true magnification values required. Increase of the mechanical tube-length simply alters the length of the *optical* tube and so proportionately increases the magnification of the objective. The results of an increase of the draw-tube are now *really* true and *not* approximations as we before stated, when we considered the matter in the earlier stages of the argument and before the difference of optical and mechanical tube-lengths was pointed out.

This concludes the explanation and discussion of the leading points concerning the methods of ascertaining the real magnification values of objectives and oculars under the old methods adopted before Professor Abbe came upon the scene with his new nomenclature. In consequence of his researches in the subject of microscopy in general, and his great and many investigations in all things optical in particular, a revolution has been slowly but steadily brought about in the minds of most computers, more especially evident since the introduction of his apochromatic series with the accompanying compensating eyepieces. The ingenuity of the suggestions embodied in the conception and carrying out of this apochromatic system and the practical utility found to exist in the general everyday use of his methods of evaluation both of objectives and eyepieces, have induced most up-to-date manufacturing opticians to adopt his lines of thought and the leading features of his nomenclature. To an explanation of this we will now turn.

The Abbe Nomenclature

We have already stated that Professor Abbe proposed the length of the optical tube for the Continental microscope of 160 mm. mechanical tube-length should be 180 mm. and 270 mm. for the English model; but we did not mention he decided to fix the position of the image plane for both models *at 12 mm. within their draw-tubes.* It is obvious this involved the construction of his compensating oculars for both models to be such that their lower focal planes should lie 12 mm. from the end of the draw-tube when slipped into their working position. This was an excellent idea, for interchange of oculars

involved practically no change of focus.[1] Further, he elected to call the initial value of every *objective*—whether for the short tube or the long—what it would be if treated as a *single simple magnifier* held close to the eye : hence a 2-mm. would be said by him to have an initial magnification of 125 diameters (250÷2) whether used on the short or the long tube. It is at once seen that here he made a pronounced departure from all convention, for it was obvious that if a 2-mm. magnified 125 on the long optical tube of 270 mm., it could not possibly magnify the same amount on a tube 180 mm., two-thirds the length. To correct this apparent anomaly he gave to his oculars fictitious values, in other words *not* magnification values such as would be obtained by the hitherto orthodox method of dividing 250 by their focal lengths. He consequently called his fictitious figures by a new name, that of "angular magnifications," the actual quantities being obtained by employing the following ingenious though absolutely arbitrary method.

He considered, as we have already said, that the objective was nothing but a simple magnifier, of a power corresponding to its focal length, producing a large and distant image of the object. Thus the "initial" magnifying power of all his objectives comes really to depend upon *their focal length only*, and *quite independent of the length of the draw-tube.* In accordance with the conventional distance of distinct vision the value, as we have said, was based on a 10-in. or 250-mm. vision distance. Dividing this by the focal length gives the initial power of the objective according to his system.

The distant *image* produced by the objective can, like any other distinct *object*, be made to subtend a *larger angle* by looking at it through a telescope, and according to the power of such we should naturally get more or less "angular magnification." Of course, microscopes are not made up of a magnifier plus an astronomical telescope ; but Professor Abbe got over that difficulty by *imagining* a compound lens placed at the upper focal plane of the objective, such lens being composed of a concave portion just the right power to weaken the objective so as to turn the real projected image into a distant virtual

[1] Messrs. Bausch & Lomb, of Rochester, N.Y., The Spencer Lens Co., of Buffalo, N.Y., and some other makers adopted the same for their ordinary Huyghenian eyepieces in pre-war times.

one, in contact with an exactly *compensating* convex lens which formed the *imaginary* objective for his *imaginary* telescope.

The numbers then on the compensating oculars mean the angular magnifying power of the imaginary telescope, with its imaginary but *otherwise perfect* objective in the upper focal plane of the microscopical objective. Obviously this telescope gets longer when the tube-length is increased, as on a real telescope, hence the same eyepiece magnifies according to the length of such telescope. Professor Abbe ideally pictures two of these telescopes, one with an imaginary object glass 180 mm. focal length and the other with one of 270 mm. in length. It now becomes evident that the ocular must apparently magnify 1·5 times as much on the 270-mm. telescope as on the 180 instrument, and that explains the reason why he tells the microscopist if he uses the short-tube eyepieces on the long-tube microscope their values must be considered 1·5 times greater.[1]

[1] As the above paragraph may be difficult to understand, perhaps the following may make it more clear: Let Fig. 97 be considered: a microscope consisting of an objective O, field-lens F, and eye-lens E is turned

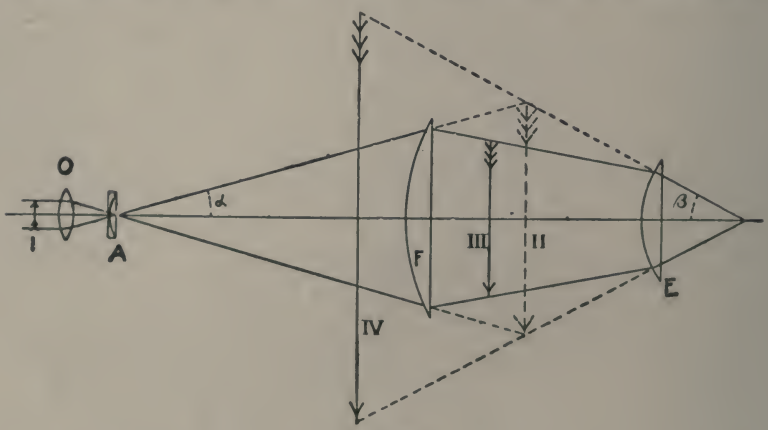


Fig. 97.

on an object I. The objective *alone* would form the real image II. This is intercepted by the field-lens F and changed into the smaller, but sharper, *real image* III, which latter is magnified by the eye-lens E into the *virtual* image IV seen by the eye. Abbe's imaginary compound lens is placed at A, in the upper focal plane of the objective—*where any lens may be placed without altering the equivalent focal length of O*—and is supposed to be

It will be readily seen in Abbe's nomenclature the initial values of the objectives are *not* powers of magnification at all in the strict and ordinary sense of the word, for they will not be found correct if experimentally tested on a 160-mm. or on a 250-mm. *mechanical* tube instrument in the manner previously explained, neither are the eyepieces figured with their real magnifying powers obtained in the ordinary fashion, as we have already shown. But, strange as it at first sight may appear, when these two (if we may so speak) fictitious values in question are multiplied together the result is nevertheless the true magnification required. On consideration, however, such is not to be wondered at, if the subject be looked at in a different way. There are four factors employed. The first, which we may call F, is the focal length of the objective; the second, which we may term E, the focal length of the eyepiece; the third, T, the optical tube-length; and the fourth and last, V, the conventional distance of distinct vision. In the *old* method of evaluation to obtain the final magnification we have $\text{Mag}^{n.} = \frac{T}{F} \times \frac{V}{E}$, whereas in the Abbe method we have $\text{Mag}^{n.} = \frac{V}{F} \times \frac{T}{E}$. By this it is at once seen that, after all, the factors are multiplied together in the same fashion, which explains the apparent anomaly. For example, let a 2-mm. short-tube objective be dealt with. Its Abbe value is 125. If tested on a short-tube microscope (by the methods already described) of 160 mm. *mechanical* tube-length, its magnifying power is not 125 but 90; and if on a 250-mm. *mechanical* tube-length it will be 135. Still, if we use on the short-tube instrument, say, an 8-times compensating ocular, the result—obtained in the usual manner 125 × 8—is 1000; a quite correct estimate,

composed of a concave and a convex component neutralising each other. By reckoning the *concave* of suitable focal length (equal to the optical tube-length) with the objective, the latter becomes a magnifier producing a very distant image of the object. The convex part of A, taken with the eyepiece, forms an astronomical telescope by which further "angular" magnification is obtained—*i.e.* whilst the eye placed behind the imaginary *concave* lens would see the object under the angle α, it sees it through the imaginary telescope—under the larger angle β. Tangent α divided by tangent β is the proper figure to engrave on the eyepiece, according to Abbe's nomenclature, as its power.

because *really* on the 180 *optical tube* the magnification of the objective has been shown to be actually 90 (180 ÷ 2), and the 8 eyepiece having a focal length of 22·5 mm. (which means in old-fashioned language a true magnifying power of 11·11) produces a net magnification of 999·9.

A little further thought will show that the figures for the "angular magnification" of any of the compensating oculars can always be correctly obtained by dividing 180 mm. by their focal length in millimetres for the short tube, or 270 mm. for the long.

Further, it will be now understood that as the initial values of the objectives (under this style) are recognised not to be *really* the true values either with the 160 or 250 mm. *mechanical* tube-lengths, and that as the figures for the oculars are not the *real* magnification quantities *in diameters*, why the term *fictitious nomenclature* has been by some writers applied to Abbe's elegant method, to distinguish it from what has been designated as the rational system already explained. But in justification for its general acceptance, it will be seen to be an arrangement of great convenience for the ordinary microscopist to calculate the final magnification at the eye end for the two ordinary models of microscope—simply by multiplying the two values, that of the objective and the ocular, together; always provided the *optical* tube-lengths are either 180 or 270 mm. But it is just here at this point where the system may be said to break down in convenience. If when using, say, a 4-mm., without cover-glass adjustment, an additional draw of the tube is necessary to correct an extra thin cover-glass, the optical tube-length is at once altered and the validity of the initial power at once flies to the winds. To obtain the real final magnifications under these circumstances, it is first necessary to find out the new length of the optical tube by measuring the distance from the upper focal plane of the objective to the lower focal plane of the ocular, which we have stated is fixed by Professor Abbe to be 12 mm. below the mouth of the draw-tube (let us say for argument this tube-length was found to be 200 mm.), and then, to ascertain the new values of the ocular, work a proportion sum. Seeing that the normal optical tube is 180, we say:

180 : 200 as the angular value of the eyepiece is to the required figure.

Working this out and presuming the eyepiece we were using was, say, an 18 on the short tube, we find the new fictitious value of the ocular is 20, and therefore the final value of the total magnification at the eye end will now be $60 \times 20 = 1200$ instead of $60 \times 18 = 1080$, which it would have been with the normal optical tube-length.

Owing to this little difficulty, there are some who dispute somewhat warmly the advantages "presumed to exist" in the new nomenclature; but, notwithstanding, as we have before pointed out, the method has "taken" so with nearly all up-to-date manufacturing opticians, that it will be rare in the near future to find any who do not adopt it.

With reference to its adaptation to objectives, when constructing a "series," it is not at all times easy to regulate the computations and the manufacture in such a manner as to conform strictly with the "new method." Constructively speaking, Abbe's fixed position for the upper focal plane is 32 mm. below the shoulder of the objective, because the optical tube-length of 180 mm. + 12 mm. (the position of the image plane below the end of the draw-tube) makes 192 mm., and 192 less the mechanical tube-length of 160 leaves 32 mm. as the limit. The computations, therefore, have then to be performed with such an accuracy and in such a manner that these figures shall be correct to within a *possible error not exceeding* 5 *per cent.* Looking back to the experimental examination of the 2-mm. short-tube apochromatic, we found the position of its upper focal plane to be 22·7 instead of 32 mm. from the shoulder, which is an error of 9 mm. in 180, just equal to 5 per cent. But, slightly to compensate this, and so reduce the error to about $4\frac{1}{2}$ per cent., we found the focal length 1·99 instead of 2·00.

The idea of Professor Abbe to make all his apochromatics to one standard (save with a possible error not exceeding 5 per cent.) was doubtless a very good one; but, as we have just said, at the same time it very often taxes the powers of the computer. In most cases this can be done with a fair amount of accuracy; but in some instances, when the position of the upper focal plane is *unavoidably* wrong to a greater extent than would seem allowable, the careful computer will intentionally give to his objective a focal length different from that engraved upon it, so that the final magnifications figured out according to Abbe's

convention shall work correct for obtaining the final magnification values.[1]

On the Magnification of Objects in general, and on the Limit of Useful Magnification in particular with Various Objectives

There is no doubt that to the *lay mind* the most attractive feature of the microscope lies principally in its wonderful power of magnification: it naturally seems marvellous that an instrument can be constructed that will show anything enlarged a thousand diameters. But to the intelligent microscopist magnification pure and simple is quickly found to be of very little value unless the objective with which it is obtained is accompanied with sufficient numerical aperture to render the details evident. Professor Abbe, in one of his laconic observations, went so far as to say, "Empty magnification is of no service whatever." It will be well to explain what he meant. To do so, let us, by way of a popular illustration, presume for a moment we have a picture of a house with windows, front door, and chimneys complete; and let it be further understood such a picture was formed upon some stretchable support, such as a piece of sheet india-rubber, and that its size was $4\frac{1}{4} \times 3\frac{1}{4}$. It is not difficult to understand that if this be stretched, say, to whole-plate size, $8\frac{1}{2} \times 6\frac{1}{2}$, the picture is enlarged; in other words, it is magnified two diameters. If this now be examined, we shall see no further details whatever. It is true the chimneys are larger, and so are the windows; but nothing more is shown about them with respect to detail in their structure or appearance. This is Abbe's "empty magnification." But if we had imparted to the objective magnifying the object that inherent property of adding detail at the same time that it magnifies—which is the same as saying if we had added to its numerical aperture—we should *then* have found the whole-plate picture would have been furnished with distinctly fresh particulars.

[1] We should mention that a little instrument called the Eikonometer has been devised by Professor Wright to enable the microscopist to obtain experimentally the exact value of any magnification with any objective, any ocular, and any length of draw-tube. It is described, with the method of use, in the chapter devoted to accessory apparatus.

The windows would have been seen to contain curtains, the door a knocker, and the chimneys would have shown cracks in them. Further, if we repeated the process and increased the magnification and the numerical aperture, we should find the picture provided with more details still, for now the window curtains would be seen to be of lace, for the pattern is visible, the knocker on the door would appear distinctly as a serpent coiled up into the requisite shape, whilst the chimney-pots are evidently very old ones, as they show holes and minute cracks we could never see before. This illustrates in a popular manner what we may call "full" amplification in contradistinction to Abbe's "empty magnification."

With respect to the microscope, the relative peculiarities of the different kinds of amplification are perhaps best seen by examining the two reproductions shown in Figs. 3A and 3B, Plate I. The first illustrates "empty magnification," the objective used being of insufficient numerical aperture to resolve the diatom into dots; whilst in the second, on the contrary, the objective having a higher numerical aperture their presence is seen quite distinctly, although, be it understood, the actual magnification in each case is similar. It is evident then a certain relation should exist between amplification and numerical aperture, so that empty magnification may never result, unless from some special cause such may be desired.[1] This has been fully discussed in the chapter devoted to the N.A. of objectives; but an outcome of the subject, although at first sight it may not be immediately apparent, is what may be called the converse of the problem, viz. seeing that the N.A. given to an objective is necessarily limited, to what extent is magnification justifiable by eyepiecing, so that fuzziness may not occur; in other words, *what is the limit of useful magnification?* (and upon what theoretical grounds is such obtained) in contradistinction to "what is the limit of useful numerical aperture?" Theory shows indisputably that, owing to the finite wave-length of light, the image of a point cannot possibly be

[1] When dealing with objects such as tissues and the like, "empty" magnification *may* be required simply to render the object more distinctly visible by appearing larger. Pathological objectives are sometimes made of specially low numerical aperture, for the further advantage of increasing their depth of focus.

rendered by any lens or combination of lenses as another *point*, but must be represented by a *disc* of more or less sensible diameter. It is notorious, by way of illustration, that in the case of a telescope a star—which may for this purpose be regarded as a point of light—is not seen by the eye or shown on the photographic plate as a *point*, but as a *disc*. Moreover, it is known in the microscope that the size of this spurious disc is not necessarily a fixed quantity, as its diameter depends upon three factors: (1) the numerical aperture of the objective; (2) the wave-length of the light used (or perhaps it may be more convenient to say the number of waves to the inch of the light employed); and (3) the final magnification of the image. Seeing that twice the number of waves to the inch of the light employed[1] multiplied by the numerical aperture of the objective furnishes what is called its "resolving power," we may at once state, if the "resolution" of the objective be divided by the final magnification of the object, the reciprocal of the quotient is the diameter of the disc required. Take, for example, light having 47,500 waves to the inch and an objective N.A. 1·40, and a final magnification of a thousand diameters. Simple arithmetic shows at once a circle of confusion of $\frac{1}{133}$ of an inch in diameter. We need scarcely add what we have said as applying to a *point* of light and its image produced by a lens also applies to a *line*, for that may always be considered as nothing but a sequential aggregation of juxtaposed points.

It is very readily understood that by a suitable manipulation of the factors we have just mentioned as regulating the size of a spurious disc, its diameter may be kept within almost any prescribed limit. Hence the question very naturally arises, What is the smallest permissible disc (*i.e.* what is the least

[1] Although mentioned in detail later on, it may be convenient to remind the reader here—seeing that wave-lengths are usually spoken of in the text-books in terms of tenth-metres—how to convert such measures into terms of the inch. This is easily effected by dividing the number 254,000,000 by the wave-length in tenth-metres, the result being that required. *Vice versâ*, measures in inches can be at once changed into tenth-metres by dividing the same "nine-figured expression" when the quotient is the amount sought after. If, however, the wave-lengths be expressed in terms of what is called $\mu\mu$ (double mu), the nine-figured expression must be deprived of one of its ciphers and then treated as before. Visual light then of 47,500 waves in one inch is equal to about 5347 tenth-metres, or 534·7 in terms of double mu.

amount of fuzziness that can be tolerated) so that the eye may see the object distinctly defined? Convention has agreed that the diameter of this disc shall not exceed $\frac{1}{100}$ of an inch, consequently the care of the conscientious and practical microscopist should always be exercised, when adding oculars of increasing power, that this limit of disc-diameter is not exceeded. To assist him then in carrying out this extremely important detail, as well as two other cognate problems, the three following formulæ are given:

1. The first shows the method of ascertaining the diameter D of the spurious disc with a known magnification m; an objective of a given numerical aperture N.A.; and light of a number of waves to the inch equal to L.

$$D = \frac{m}{2(L) \times N.A.}.$$

For example, with an objective of numerical aperture, 1·0; L 47,500 waves to the inch and magnification × 1000, then—

$$D = \frac{1000}{95000 \times 1} = \tfrac{1}{95} \text{ of an inch.}$$

2. This formula furnishes the means of ascertaining the magnification m that will produce a disc of given diameter D with a light (L) of specified number of waves to the inch, and an objective of given N.A.—

$$m = 2(L) \times N.A. \times D.$$

Example: L = 47500, N.A. ·65 and D = ·005 in.,
Then— $m = 95000 \times \cdot65 \times \cdot005 = 308\cdot75$ diameters.

3. If it be required to find the N.A. that must be employed to obtain a given circle of confusion D, with light of L waves to the inch and a magnification m, then we employ—

$$N.A. = \frac{m}{2(L) \times D}.$$

Example: If D = $\frac{1}{250}$ in., L = 47500 and m = 500,

Then— $N.A. = \frac{500}{95000 \times \cdot004} = 1\cdot32$ nearly.

The table following is arranged to show at a glance the limit to be placed on magnification with different numerical apertures

so as to ensure a disc of $\frac{1}{100}$ of an inch not being exceeded when white light averaging 47,500 waves to the inch is employed:

N.A.	Approximate magnification limit.
·3	285
·5	450
1·0	950
1·20	1140
1·30	1235
1·35	1282
1·40	1330

If this table be carefully studied it will soon be seen to reveal a law, by the recollection of which such an array of figures need not be borne in mind. It is this. *The magnification must never exceed* 1000 *times the numerical aperture or thereabouts.* The utility of employing blue-violet light for the most delicate work is very obvious from what has been said, and this was the reason the writer spent so much time in perfecting and arranging an apparatus for obtaining this monochromatic illumination with ordinary limelight, more especially for photo-micrography.

Seeing that N.A. may be said to have practically reached its limit, further advance then in resolving power can only be expected by the use of light *beyond* the violet end of the spectrum, which is usually called by the name of ultra-violet light.

Unfortunately, however, till lately two difficulties have seemed insuperable: one was the manufacture of a glass that would transmit these rays in question, and another was how to focus an image formed by light of this description, seeing that such is practically invisible to the human eye. The great ingenuity of the firm of Carl Zeiss has, however, after some difficulty, overcome these difficulties. The objectives are formed of molten quartz, and are corrected for a wave-length of 275 $\mu\mu$, oculars being specially constructed from quartz crystals; whilst to enable a focus to be made, a screen is temporarily employed—we believe of a character much like that used for X-ray work—by the use of which the rays are for the moment increased in length sufficiently for the eye to see the image, and hence for

this operation to be effected ; but which of course is removed whilst a photograph is being taken.[1]

It is obvious from what has been said that as the circle of confusion becomes *smaller* the *shorter* the wave-lengths of the light employed, the aim of the microscopist is to be able to command the use of light with a greater number of waves to the inch than 47,500. Monochromatic screens and films of gelatine are used for this purpose, and they are explained and described later on together with an arrangement by the author for obtaining blue-violet light.

[1] The special features of this new type of objective are a very perfect union of rays (spherical correction) for the special light selected, the lack of a correction of chromatic aberration, and the composition of the system from uncemented single lenses all being formed out of the same material. It is needless to point out as there is no chromatic correction at all (because such is not needed) objectives of this make can only be employed with monochromatic illumination of the special wave-length for which the computation is designed.

CHAPTER VIII

THE SUBSTAGE CONDENSER AND THE DIFFERENT CONES OF LIGHT; AND THE SUBSTAGE DIAPHRAGM: ITS ABUSE AND ITS USE

A SUBSTAGE condenser consists of a system of lenses which collect the light from the illuminant and concentrate it upon the object. The apex of the illuminating cone impinging upon the specimen without the intervention of any stop or diaphragm is said to form a "solid cone of light." If an iris or other diaphragm contracts the aperture of the lenses, the base of the cone being thereby reduced in diameter, the cone is spoken of as "a narrow one." If a stop be placed at the *base* of the cone, so as to prevent all rays passing through the *centre* of the back lens of the combination, the object has then a hollow cone of light impinging upon it, and is said to be illuminated by "annular light." See page 376 and Addenda.

These condensers, or illuminators as they are sometimes called, vary in focal length as well as numerical aperture, and are chromatic and achromatic in their corrections, a third class being dubiously called apochromatic.

Several different forms of *chromatic* condensers have been made from time to time, but many of them are inferior to that devised by the late Professor Abbe and made by Carl Zeiss which has gradually supplanted them; hence we confine our remarks to this special form of substage condenser.

In its simple low-power form it is composed of a collecting lens and a hemispherical front which forms the focus of the light upon the specimen, the system having an aperture of not more than N.A. 1·0. If this be well made it answers its purpose, but unfortunately as supplied by some other makers, it often leaves a *great deal to be desired.* It is this fact that has led many microscopists (unfortunately including ourselves) to speak disparagingly of the performance of this combination; but of

course, as its name implies, it does not profess to be achromatic, and hence, when used in its high-power form next to be described, shows colour to an objectionable amount when employed with oblique light. The high-power form, N.A. 1·30, has an additional lens, and requires oiling to the slip in the same manner and for the same reason as an objective, of the immersion type, requires oiling to the cover-glass.

Each of these chromatic condensers is mounted in a cell which drops into some sort of sleeve forming part of the substage. It is then capable of being racked up and down as necessity demands. In many Continental forms of stands this sleeve forms part of an independent fitting which is fixed—though not permanently so—to the substage, and is capable of being turned aside out of the way when not required.

The *achromatic* variety, even for visual purposes, especially when oblique light is being used, is in our way of thinking far superior, and we always recommend it. In photomicrography too, *unless monochromatic light be used*, achromatic condensers are to our mind of greater service than the less complicated variety. It is perfectly true, however, that Mr. Poser, late manager of the English branch of Carl Zeiss in London, who was well known both as a scientific expert of the highest order and as a highly practical mechanician, has taken photomicrographs with the chromatic condenser in use, which are pictures beautiful to behold ; still we have heard the critic say, " Would they not have been even better (if possible) had an achromatic form of illuminator been employed ? " Be this as it may, bearing in mind how differently an expert uses a piece of apparatus to one who is merely a tyro, we think the general tendency amongst all microscopists is to prefer the more highly corrected condenser, even for visual purposes. This statement seems evidenced by the fact that most of the modern condensers are of the achromatic variety, such as those by Beck, Swift, Watson, and later still by Zeiss, Leitz, as well as others ; but it must also be stated there are few Continental firms who advance their claims with much enthusiasm.

The *apochromatic* condenser is a name unfortunately adopted by some opticians for an otherwise good combination, especially those by Powell & Lealand, and recently by Swift. It is evident, however, that such is a misnomer, seeing that an

apochromatic combination must before all things be absolutely free from spherical aberration and spherical zones, whilst all these condensers are not even capable of giving a full aplanatic cone! Notwithstanding this we readily admit the type is of a useful kind (although not better than the first-class achromatic), as we ourselves can testify, having employed one in photo-micrography in many instances with considerable satisfaction.[1]

The leading points to be considered in a condenser are: the focal length, numerical aperture, size of the aplanatic cone, and the definition.

With respect to the focal length of any given condenser, this is usually furnished by the maker as a result of his computations, and it is difficult to obtain experimentally save but by the same method as we have already given when dealing with a similar inquiry in connection with ordinary microscopic objectives. In the case of condensers made in England, seeing they are small and mostly mounted in the "universal fitting," that is easily performed by screwing them on to the nosepiece, and proceeding in the ordinary way; but this cannot be carried out with those made by Zeiss and other Continental firms on account of their great size. The only method we know is to measure the size of the illuminant (taking care such shall be small by interposing a diaphragm in front of the same), and focussing and measuring it carefully. This measurement should be compared with the size of the same aperture as indicated by other condensers of known focal length under similar conditions; but the method is not a satisfactory one, and requires carrying out exceedingly carefully, or false results may be obtained.

[1] One point we ought perhaps to mention, seeing it has been brought forward as a further argument why the so-called apochromatic condensers cannot be truly apochromatic, is the entire absence of any compensating ocular or its equivalent. This at first sight appears a very formidable and convincing reproach. On consideration, however, it is not so because the important part played by the compensating ocular is mostly effective in the outer areas of the field of view, and least so at the centre; hence it would only be with extreme oblique light such might be appreciable. But even here the matter is of no consequence in the case of condensers, as the loss of refinement in detail brought about by the non-adjustment for chromatic differences of magnification (the function of the compensating eyepiece) is not appreciable to the slightest degree; hence the entire objection falls to the ground.

With regard to the most suitable focal length for condensers, so as to suit objectives of different powers, a limit is placed thereon which may not be immediately apparent. For that reason we will enter into the matter somewhat fully, more especially as the subject has escaped the attention of most writers in the text-books published.

It will be noticed by looking through the list given on page 152, that the focal lengths of illuminators made by different opticians become less and less within somewhat defined limits the higher their numerical aperture. To explain this involves a little close attention. Consider a highly apertured condenser, say one of N.A. 1·35. It has to fulfil two conditions: to furnish plenty of light, and to have a high numerical aperture. Now the amount of light—which is another way of saying the brilliancy of the image of the illuminant—in any lens is augmented by decreasing the ratio of focal length to aperture. Readers who are familiar with photography will recollect that $\frac{F}{8}$ supplies four times the amount of light to the sensitive plate that is afforded by $\frac{F}{16}$, no matter what is the focal length of the system in use. This arises, as the reader also full well knows, because the available diameter of the lens is twice as great, and as the area is the square of the diameter so the *quantity* is four times as much.

Now to obtain the value of any numerical aperture expressed in terms of what we may call the F ratio, for reasons set forth in the footnote,[1] we must divide ·5 by the numerical aperture in

[1] (1) N.A. always $= \frac{\frac{1}{2}d}{F}$, where d is the diameter of the back lens and F the focal length; for example:—if 1 in. be the diameter of back lens and 4 in. the focal length, we have

$$\text{N.A.} = \frac{\frac{1}{2}}{4} = \frac{1}{8} = \cdot 125.$$

(2) Rapidity or F ratio $= \frac{F}{d}$, in our example $= \frac{4}{1} = 4$, called $\frac{F}{4}$.

To establish a ratio between the two quantities, multiply both sides of (1) by F and divide both sides by d. Then we obtain—

$$\frac{F}{d} \times \text{N.A.} = \tfrac{1}{2};$$

and further dividing by N.A. we find—

$$\frac{F}{d} = \text{F ratio} = \frac{\frac{1}{2}}{\text{N.A.}} \text{ or } \frac{\cdot 5}{\text{N.A.}}.$$

question, the quotient being the denominator required; thus N.A. 1·40 = $\frac{F}{\cdot 357}$.

For convenience of reference the following table is set out :—

$$1\cdot 40 = \frac{F}{\cdot 357}\cdot$$
$$1\cdot 35 = \frac{F}{\cdot 37}\cdot$$
$$1\cdot 00 = \frac{F}{\cdot 5}\cdot$$
$$\cdot 95 = \frac{F}{\cdot 52}\cdot$$
$$\cdot 65 = \frac{F}{\cdot 77}\cdot$$
$$\cdot 30 = \frac{F}{1\cdot 6}\cdot$$
$$\cdot 24 = \frac{F}{2\cdot 08}\cdot$$

By this it is seen that the 1·35 condenser really works, as the photographer would say, at $\frac{F}{\cdot 37}$; in other words a very brilliant image of the illuminant is rendered, one exceedingly so in comparison to that formed by a lens, the aperture of which is restricted to $\frac{F}{8}$, which only reaches ·0625 in terms of numerical aperture. In the manufacture of any condenser for the microscope, however, a limit is placed upon the diameter of its back lens, because otherwise the combination would not fit into the "universal thread," which is the standard size mostly adopted by English opticians; and if larger than this, complications are found to arise with the working of the centring arrangements. This actual limiting diameter is about $\frac{6}{10}$ of an inch. But to obtain these high numerical apertures large back lenses to the combination are absolutely necessary, far larger indeed than it might be supposed, because it is the ratio of the *semi*-diameter of the back lens to the focal length that constitutes the numerical equivalent. Hence, as a limit is imposed on the computer in this direction, he must necessarily lower the focal length accordingly, and consequently we find the actual focal length of these high-apertured combinations must be somewhere about $\frac{1}{4}$ or $\frac{1}{5}$ of an inch to fulfil all the conditions of which we have spoken. The microscopist then has not much scope for selection.

It can be readily understood that the light issuing through the 1·35 condenser of so short a focal length (seeing that the shorter it is, the smaller the image of the illuminant) is exceedingly intense, which can be better appreciated by the photographer when we remind him the F ratio is so remarkably small. It will be further seen that the 1·35 combination passes about 18½ times the light transmitted by a low-power condenser, say one of N.A. ·3, by which we mean the actual image in the one case is about 18½ times brighter than in the other. A question may here be asked which is often met with. If this theoretical statement be true, how is it that when using the ·3 condenser and an inch objective N.A. ·3, say with an ocular × 4, the light of the lamp is almost unendurable ; whereas with the 1·35 condenser, and, say, a $\frac{1}{12}$th of corresponding aperture with ocular × 8, the field is sometimes too dull to be useful? How can this be when the higher apertured condenser, we say, passes through 18½ times more light? It is due to the difference in the relative final magnifications used. In the first case this amounts to only 40 (10 × 4), whereas in the latter it is 1000 (125 × 8). The direct consequence is that the light from the lamp is *really*, although not apparently, spread over a very much larger area of field (only a small piece of which is utilised), and hence is *fainter to the eye* than in the previous case, where, owing to the amplification being so much smaller, the image, though primarily of much less brilliancy, is so much less spread out *that to the eye it actually appears very much more intense.*

It is evident then that the choice of focal length for condensers to be used with objectives of different numerical aperture is necessarily limited by the demands of optical construction. Without pressing the matter further, these conditions limit the focal length of the illuminators for high aperture to about $\frac{1}{5}$ to $\frac{1}{4}$ of an inch ; for those about N.A. 1·0 to $\frac{2}{3}$ to $\frac{2}{5}$, whereas with quite low powers it may stretch between $\frac{2}{3}$ of an inch to even 2 in.

From what has been said, the image of the illuminant in high-apertured systems is necessarily small. Usually the diameter afforded is sufficient, but at times it is often felt that a larger flame image would be very useful. This can be effected by use of the bull's eye, as we have already stated, but its use is not always attended with desirable results as respects definition ; hence, to meet this end, Mr. Conrady has computed and Messrs. Watson

& Sons have made a condenser of N.A. 1·0 with aplanatic cone of ·95 that has a trifle longer focal length and hence furnishes an image about $\frac{1}{3}$ times greater. Of course this demands a large back lens, but its diameter just enables it to be used with a special centring fitting in the ordinary substage. It is a very excellent combination, and by removing the top lens the remainder can be employed for objectives of lower numerical aperture.

There are several makers of good condensers, but of course many microscopists have their favourites; before selection, however, it would be well for the student to test the performance of the combination in the manner about to be explained. First, as respects its **numerical aperture.** This can be often obtained by the use of the apertometer, but not if it be a condenser with a large back lens. It is best then to use the method described on page 97, first suggested by Mr. Conrady.

But condensers are now more usually classified according to the N.A. of what is called their "aplanatic cone," a term which we at once proceed to explain.[1] This is one often used but rarely explained on account of the somewhat involved nature

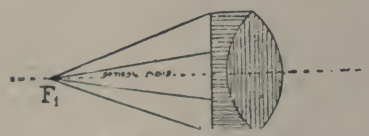


Fig. 98.—Aplanatic.

Fig. 100.—Uncorrected Lens.

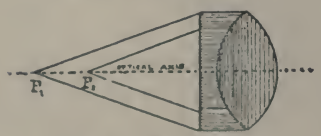


Fig. 99.—Over-corrected.

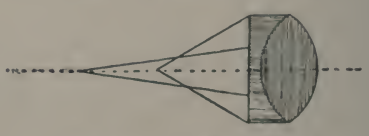


Fig. 101.—Under-corrected.

of the reply. The word itself, derived from the Greek, means, in point of fact, "free from wandering," by which the optician understands (as he uses the word) that all rays, whether from the periphery of the lens or nearer its axis, *shall meet in one*

[1] This term, introduced some time ago by Mr. Nelson, may not be considered by some a very happy one; indeed, it has been thought that to have altered it to "efficient cone" instead would have been preferable; but the term is so firmly established in its conventional meaning that we hesitate to change it.

point in a given plane, as shown in Fig. 98. This is the ideal perfection of the optician's art. As an ordinary uncorrected lens always suffers from what is called "spherical aberration" (by which is meant the marginal rays come to a focus at a point on the axis closer to the lens than those situated nearer to its axis or centre, as seen exaggerated in Fig. 100), so the art of the optician referred to is to try to unite these planes of focus by combining glasses having different properties. If he overdoes it, producing what is technically called "*over*-correction," he brings the peripheral rays too far along the axis, as shown in Fig. 99; and if he does not correct enough—"*under*-correction," as it is termed—he leaves the combination with the same error outstanding, although in a less degree, as that possessed by an uncorrected lens, as shown (greatly exaggerated) in Fig. 101.

Aplanatic Cone.—To ascertain the size of the largest aplanatic cone of a given condenser, say one of N.A. 1·0, we proceed as follows: Fix it in the usual position on the substage, and place on the nosepiece of the microscope first an objective of N.A. ·6, and on the stage a diatom. Focus it with the objective, using as an illuminant the edge of the flame, and rack the condenser up and down until this image of the flame is seen across the field with the diatom lying in its centre. This is obtaining what is technically called *critical light*, and the resulting image is called "*the critical image*." Shift the diatom just out of the field of view, still leaving a portion of the slip and its cover-glass *in situ*. Remove now the eyepiece, and look down the tube of the microscope. One ought to see the back lens of the objective full of light, because the aplanatic cone of the condenser should be greater than that of an objective 0·6, such as we are supposed to be here using. Return now the eyepiece and remove the objective, substituting one of 0·95 N.A.; again focus the diatom, and again obtain critical light by focussing the condenser on the diatom until the edge of the flame is seen across the field. Once more shift the diatom out of the field, and look down the tube as before. The back lens should be quite evenly filled if the aplanatic cone equals the numerical aperture. Then close the iris diaphragm until its edge is just seen, and carefully note the exact size of the opening with a pair of compasses. Now remove the 0·95, and place in its stead an immersion objective

of 1·40 N.A. Treat as before, with respect to focussing and obtaining critical light, and look down the draw-tube. Only the centre two-thirds of the back lens is now seen full of light, and the slightest touch of the condenser upwards, so as to try to fill the lens to a greater amount, will cause two dots of black to appear on each side of the lamp flame, which then becomes immediately recognisable. The last point before the appearance of these black dots (really due to spherical aberration) indicates the largest aplanatic cone of the condenser. Slowly and cautiously close the iris diaphragm until it is just visible, and measure the size of the aperture with compasses, as before. If the diameter is slightly greater than the previous measure for the 0·95 lens, the aplanatic cone is, of course, just above 0·95. A little experience and thought will soon render these operations quite easy, and the microscopist will be able readily to compare the largest aplanatic cone of the condenser he is testing with its advertised N.A., and the performance of one condenser with another.

The difference which so often exists in these two measures is very striking and is said to be mostly due to errors in spherical aberration, most condensers being more or less under-corrected (Fig. 101), and consequently focussing their *central* rays at a greater distance than their *peripheral* ones. If a condenser be well corrected, the lamp-flame image, as seen on looking down the microscope with the eyepiece *in situ*, should be, when accurately focussed, intensely bright, whilst the field is commensurately dark ; but very frequently this darkness is conspicuous by its absence.

It may be here asked, What does it matter even if the condenser should be badly corrected and possess a small aplanatic cone? It is this. The object of a condenser is to bring as much of the light of the illuminant as possible to a focus on the object. If now all the rays do not come to the same focus all of those which come to another are so many lost, and only serve to scatter light into the field ; and besides this, when using a broad illuminant (such as is produced after obtaining critical light with the edge of the flame, by turning the lamp-flame broad side on), not only is there an unequal illumination of the field which is immediately apparent, but no critical light is obtainable at the margins of the field without losing it in the

centre, and *vice versâ*. Hence one of the uses of having a large aplanatic cone is to obtain uniform lighting.[1]

As now the best of definition will only be over the area where critical light exists, so a critical image cannot be obtained over *the whole field at one and the same time*, and appearances may thus be produced in that part where it is absent, which to say the least are objectionable.

The **Definition** afforded by a condenser is important, but only up to a certain extent. If a good sound image of the flame edge is not well shown it is very obvious it is more difficult to obtain *critical light* with the same ease as if it were good, but condensers never give so fine images as objectives—it would make them too costly. To *test* for definition the condenser may be placed on the microscope, and its performance compared with that of an objective of the same N.A., always remembering that the slide should be turned round the opposite way, *i.e.* with the cover-glass *towards* the mirror and the plane glass of the slip *towards* the condenser when fixed on the nosepiece. This, of course, is necessary because the correction has been made, or should have been made, by the optician with respect to the thickness of the slip just as he makes the correction for the objective with respect to the thickness of the cover-glass.[2]

Another point comes into notice here. Slips unfortunately vary very greatly in thickness, some being much thinner than others, hence when the condenser, if it be over N.A. 1·0, is made for a *thick* slip and used on a thin one, one or more cover-glasses, oiled together and to the slip and condenser, must be used to fill up the gap, or else the oil will leave the slip should the condenser have to be lowered to obtain critical light. If the oil-condenser be made for a *thin* slip, it cannot be used for a thick one, and must have its top lens removed and replaced by another constructed for the purpose. We have

[1] This is the object of looking down the tube of the microscope to see if the condenser be properly adjusted before commencing to make an observation—a method we always recommend, especially when employing high-power objectives—because, if the field be not uniformly illuminated, the best results of definition are not to be obtained.

[2] The substance of these last few pages has been taken, by permission of the publishers, from the Author's book on *Photomicrography*.

three top lenses to one of the high-angled condensers we use.

With dry condensers, a plan adopted by Mr. Conrady in his N.A. 1·0 type is highly commendable, the top lens being adjustable by means of a correction collar in the same way as dry-power objectives of high power are fashioned. By this means different thicknesses of slip are provided for.

The following table, extracted from Carpenter on the microscope, of the performance of different condensers will be of interest :

Condenser.	Total aperture N.A.	Aplanatic aperture N.A.	Power.
1. Powell & Lealand's dry achromatic (1857)	·99	·8	$\frac{1}{6}$
2. „ „ top lens removed .	—	·5	$\frac{1}{3}$
3. „ „ bottom lens only .	—	·24	$\frac{2}{3}$
4. Swift's achromatic	·92	·5	$\frac{4}{12}$
5. „ „ top lens removed .	—	·22	1
6. Abbe's chromatic (3 lenses) (1873) . .	1·36	·5	$\frac{1}{3}$
7. „ „ top lens removed . .	—	·3	$\frac{2}{3}$
8. Powell & Lealand's chromatic (Abbe's formula)	1·3	·7	$\frac{1}{2}$
9. Powell & Lealand's oil achromatic (1886)	1·4	1·1	$\frac{1}{6}$
10. „ „ used dry . . .	1·0	·8	$\frac{1}{6}$
11. „ „ top lens removed .	—	·4	$\frac{4}{10}$
12. Abbe's achromatic (1888). . . .	·98	·65	$\frac{1}{2}$
13. „ „ top lens removed .	—	·28	1
14. Powell & Lealand's low-power achromatic (1889)	·83	·5	$\frac{2}{3}$
15. Powell & Lealand's apochromatic (1891).	·95	·9	$\frac{4}{10}$
16. Zeiss's "aplanatische Lupen" large field (Steinheil formula)	—	·32	1
17. Beck's achromatic dry (1883) . . .	1·0	·9	$\frac{1}{4}$
18. „ oil achromatic (1900) . . .	1·4	1·3	$\frac{1}{4}$
19. Swift's apochromatic dry (1892) . .	·95	·92	$\frac{1}{4}$
20. „ panaplanatic dry (1897) . .	1·0	·93	$\frac{1}{4}$
21. „ „ oil (1898) . .	1·4	1·30	$\frac{1}{4}$
22. Watson's parachromatic dry (1898). .	1·0	·95	$\frac{2}{7}$
23. Watson Conrady oil	1·33	1·25	$\frac{1}{4}$
24. Zeiss oil achromatic	1·30	—	—
25. Baker's semi-apochromatic . . .	1·0	·95	$\frac{1}{3}$

"The values of the first sixteen and of Nos. 22, 23, and 25

have been obtained from actual measurements; the others are from the estimates of the makers.

"The limit given in the table is for the edge of the flame as a source of light. When, however, a single point of light in the axis is the source, the condenser will be much more sensitive, and a lower value for the aplanatic aperture than that given in the table will be obtained. But as a single point of light is seldom, if ever, practically used in microscopy, it was deemed better to place in the table a practical, rather than a theoretical, and probably truer result."

The actual construction of the different condensers sold by the numerous opticians of the present day is so various that it would occupy too much space to illustrate them; but the main feature in the English-made combinations is that they are of so much smaller diameter than the Continental, which are in our way of thinking uselessly large. The convenience of having them so much smaller is very great, as quite a small substage is all that is necessary; whereas with the Continental design everything beneath the stage has to be specially arranged. It is a great pity that the size of the two different types of condensers—the English and Continental—cannot be made similar, for then interchange of condensers could be much easier effected than it is at present.

The Substage Diaphragm: its Abuse and its Use

The substage diaphragm in olden days used to be nothing else than a metal wheel perforated by many different-sized holes; nowadays it is nearly always of the iris or self-closing pattern. The diameter to which this arrangement is closed has a very far-reaching effect, and indeed is of such importance that some little attention must be given to the matter.

The first point to be mentioned is its *limiting* powers upon the working aperture of the condenser with which it is used. To understand this it is only necessary to remind the reader that the numerical aperture of any lens is practically the ratio of the available semi-diameter of its back lens to the focal length. Hence it is easy to infer that curtailing the back lens—that is, the one nearest the mirror—promptly lowers the numerical aperture of the system. But, from what has been

said elsewhere, this is obviously a serious matter, for curtailing the N.A. of the condenser involves a curtailment of that of the objective in use with it also. Here an error has crept in to which we must at once refer. One often hears it stated that, if the iris be shut so as to produce a given numerical aperture to the condenser, the objective is *always* reduced to a *similar amount;* so that, if the illuminator in question were cut down to, say, N.A. ·65, no matter the aperture for which the objective was designed, it would be cut down to the same extent. This, however, is not exactly true, though so frequently stated, and we call attention particularly to the fact because it explains certain little matters without any difficulty which otherwise are not easy to understand. Owing to a certain portion of the light coming from the condenser upon an object being diffracted by it and scattered, a small quantity of this scattered light falls upon the front lens of the objective. If now the objective system to which this belongs be one of *higher aperture than that of the condenser in use*, the extra amount falling upon the front lens is caught up by it and transmitted through the entire combination, filling the back lens to a *greater* degree than would be at first sight anticipated, and so makes the *working* aperture of the objective higher than that of the condenser itself at the moment. Although this increase in illumination, it is true, is somewhat feeble in comparison with the direct beam, still the point to bear in mind is that the back lens is filled with rays of a wider cone, which give to the combination a higher *working* aperture than is furnished by the illuminator itself. In order to ascertain the amount an objective is thus increased from this cause, in other words to be always able to estimate the working aperture of *any lens of high aperture when used with a condenser of a lower numerical equivalent*, it is only necessary to add together the two apertures and take the mean. This mean is the estimate required. Take, for example, a lens of 1·40 with an illuminator of 1·0. The sum is 2·40, and the mean 1·20. This is the working aperture of the 1·40 objective when used in conjunction with a 1·0 condenser. It is this increase we wish to call attention to, for it immediately explains why, when using a 1·40 lens, say, with a 1·30 condenser cut down to N.A. 1·0, better resolution is obtained than when employing an objective of the *same reduced numerical aperture*—a fact well known for

years to the practical microscopist. These additional rays to the 1·40 objective we speak of can be easily seen by looking at its back lens when in use with the condenser reduced to N.A. 1·0 in question. A ring of faint light surrounds the brilliant direct beam, which is entirely lost when the objectives are changed and the N.A. 1·0 substituted, the brass mount of the latter and the different shape of the lens-front preventing its admission. It is scarcely necessary to add, the object must be focussed and the objective oiled to the cover-glass in making the experiment.

So far we have only pointed out how the diameter of the iris affects the numerical aperture of the condenser, and so indirectly that of the *objective* in use at the time. We must now explain what the general effect of lowering the aperture of the condenser has upon the actual *image of the object.* This means, of course, the effect produced by using a small solid cone of light in contradistinction to a wide one, for example between using a wide solid cone of N.A. 1·30 (with a suitable objective) and a reduced one of, say, N.A. 1·0 *whilst still employing the same magnification.* The evil of this is nothing actually new, for Mr. Nelson has in his practical manner called attention to the danger of using small cones times without number, but what follows puts the matter in perhaps a somewhat different light.

One of the effects of reducing the aperture of any lens whilst employing the same magnification is to increase very sensibly what is called "the circle of confusion." We have elsewhere pointed out that owing to the infinite wave-length of light, the image of a mathematical point cannot possibly be another mathematical point, but must be a diffused disc of more or less sensible size. We have shown, too, that the usually accepted limit to the diameter of this unavoidable disc is $\frac{1}{100}$ of an inch—that is to say, $\frac{1}{200}$ of an inch on each side of the object—which, for argument's sake, we may speak of as a point. The question now before the reader is to show how lowering the diameter of the iris increases the diameter of the disc if the same magnification be maintained.

To ascertain how to calculate the diameter of the disc, only two things are necessary: (1) to find the resolution, and (2) to multiply such by the magnification. Take the first. This is obtained by Abbe's well-known law, "Multiply twice the number

of waves to the inch of the light employed by the numerical aperture of the objective." As an example, let us say the light used has 47,500 waves to the inch ;[1] twice that is 95,000 ; and so, if the N.A. be 1·40, the resolution is 133,000, which means any two lines at this distance apart can theoretically be separated if oblique light be used. Presuming now that the magnification is 1,000 diameters, then the circle of confusion—

$$\frac{1}{133000} \times 1000 = \frac{1}{133} \text{ of an inch.}$$

Should the diaphragm be further closed to N.A. 1·0 the circle is increased, whereas if finally closed to N.A. ·50 the circle is enlarged still more. The consequence of this is immediately apparent, for the object is distinctly fuzzy. Of course this circle of confusion about the point of light is really produced by diffraction phenomena ; hence as the diaphragm is more and more closed with objects of sensible area the image becomes crowded with all manner of diffraction effects, so much so, indeed, one scarcely knows what is real and what is false. White lines may appear around bacteria to "make believe" they have discrete capsules ; hairs may appear doubled-tipped, divisional markings between portions of a diatom may grow to such an extent as to appear several times thicker than they should, actually encroaching upon the true structure of the valve. To explain why this is so, why such different appearances are produced by the same cause, is very difficult to say, but it must here suffice to add that all diffraction effects (more especially, perhaps, of this type) are caused by interference phenomena between the rays coming from the *same* source of light, one with the other. Those that come in contact in the *same* phase—in other words, those of simple multiples of the same wave-length—are additive, and seem to strengthen one another, causing increased brightness ; whilst those not in the *same* phase, being perhaps half, or any portion, of a wave-length ahead or behind the others, serve to *quench* each other, causing darkness.

Diffraction phenomena then, taken as a whole, are charac-

[1] The ordinary wave-lengths of different coloured light are usually given in tenth-metres. To convert these into terms of the inch, divide 254,000,000 by the number in tenth-metres—*vice versâ*, if in inches, divide the same 9-figured quantity by the number in inches. For example, say, 5500 tenth-metres = 46,182 waves in one inch. See Addenda.

terised by what are termed "bands of brightness" and "bands of darkness," which can be understood from what has been stated; but they may also be described as being arranged in *maxima* and *minima*. These can be readily seen around the so-called point of light witnessed in the telescope when focussed on a star. It is surrounded with rings of brightness and rings of darkness, first one and then the other.[1] Now the same arrangement of phenomena, it may very justly be assumed, takes place about the image of an object other than a star or point of light, such as one would meet with in the microscope; hence it has been thought that the reason these phenomena appear to vary in appearance—sometimes a white line around an object, whilst on another occasion an increase of darkness in the dark places already known to be existent—is because from some cause hitherto unexplained—*possibly* a remote effect of contrast—the eye in one case recognises a maximum, and in the other a minimum effect; in other words, at one time sees *a bright band*, and at another *a dark one*. It should be mentioned, however, that it is quite possible the change of effect may only be due to a slight alteration of focal adjustment.

We have now pointed out the dangers of closing the iris, and what care must be exercised in so doing; it remains now, on the other hand, to be explained, strange as it may appear, how this closing effect under certain circumstances becomes of the very *greatest possible service*. We refer to the use of the diaphragm in cutting off stray light from entering the tube when employing a condenser *of higher aperture than the lens with which it is in use*—say, for example, a ·65 objective and a N.A. 1·0 illuminator. Let both be placed on the microscope. Having focussed a specimen—say an Abbe test-plate—let the ocular be removed, and whilst looking down the tube the iris be shut until its leaves just appear at the periphery of the back lens of the objective. The ocular being returned, the plate should be carefully watched whilst the iris is suddenly opened: a rush of light will be seen flooding the whole field, spoiling very sensibly the general definition of the lines. From its submerging effect on the image, this used always to be called "flooding the specimen with light." Repeating the process,

[1] In making the experiment it is well to use a monochromatic screen, placed between the eye and the eyepiece.

but on this occasion watching what happens in the back lens when it is looked at, instead of the specimen through the ocular, we shall see, when the iris is opened, that quite a quantity of stray light enters the tube—coming in edgeways through the lens—which is reflected off its edges into the ocular, causing the flooding in question. If now the iris be shut, so as to prevent this, the objective performs under fair conditions. Attention is especially called to this fact, as otherwise a combination may be blamed for a bad performance which was simply due to this irregular addition of stray light, which might not have occurred if the condenser had been of exactly the same aperture as the objective.

No microscopist then can handle his substage diaphragm carelessly, for if he does so he may produce effects that may lead him quite astray in forming an opinion upon the true structure of an object. It is not an easy matter to furnish the student with any distinct limit to this closing of the iris that is justifiable, one that will not introduce these irregular diffraction phenomena; but taken as a general rule for daily practice,

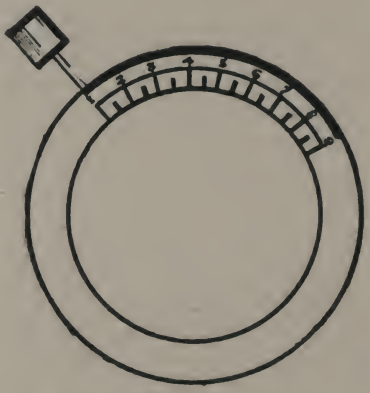


Fig. 102.

however, it may be asserted, with a moderate amount of certainty, that no objective should ever be cut down more than by, say, its outer third, by which we mean the outer third of the back lens of the combination as seen by looking down the tube of the instrument, the eyepiece being removed. If it requires more cutting down than this before a good image is

obtained—save perhaps under some peculiar circumstances—the objective is probably a valueless one, and the sooner it is got rid of the better.

Before quitting the subject of the iris diaphragm, it may be mentioned the frame in which the leaves work is often divided as shown in Fig. 102. This is for the convenience of noting *approximately* the different apertures that correspond with definite amounts of numerical aperture. These quantities are ascertained by using objectives of known values of N.A., the leaves of the diaphragm being shut down so that they just touch the periphery of the back lens of each objective (as seen when looking down the tube the ocular being removed) when in turn it is placed upon the nosepiece. The exact reading of the figures being noted in each case, when in future the diaphragm is shut down to the same figures, it at once indicates very closely the amount of N.A. in operation at the moment, no matter what objective is in use at the time.

CHAPTER IX

METHODS OF ILLUMINATION

MOST objects are examined by transmitted light, the rays of the illuminant being reflected by the mirror through the condenser, object, objective, and eyepiece to the eye of the observer. Occasionally, however, it is desirable to do away with the mirror altogether, and, setting the instrument horizontal and raising the illuminant a convenient height, to employ the light straight away from the lamp. There is little to be said in our opinion in favour of such method (which of course *cannot* be employed if fluids are used on the stage as media for objects) save but for one purpose, and that is to do away with the double reflection of the light from the mirror, owing to the glass of which it is made being too thick.[1] Some microscopists think better definition is always obtained by the use of this "direct" light, as it is called, than by employing the ordinary "reflected" beams off the mirror; but as to this there does not seem a universal agreement of opinion.

When using the lamp and mirror in the ordinary fashion, it has been already stated the light should be placed and the condenser so adjusted that the flame image is seen in the field of view of the ocular stretching across it. When condensers were first used, however, before any theory explained their use, they were racked up and down, that is to say within and without the focus, so that the flame image was *avoided*. The "Carpenter" school declared the better effect was always obtained by racking *without* the focus; the "Quekett" student maintained it was just the reverse; whilst Sir David Brewster thought the source of light should be focussed on the object, the same opinion in fact that has been held now for some time.

[1] The late Dr. Dallinger, whose great experience always demands a corresponding consideration, is said to have been in favour of using a right-angled prism instead of a mirror, the total reflection doing away with the possibility of all double images. We have ourselves found the light remarkably pure and particularly pleasant to use, but the arrangement is cumbersome to handle.

In later years still, owing to the researches of several mathematicians and others, a good deal of controversy has re-arisen upon this subject; but a series of considerations by Mr. Conrady, although arousing some opposition at first, has taken hold with microscopists, for it would appear, upon mature consideration, his arguments are indisputable. These lead us to believe that the actual focus of the illuminant is not *per se* really *an actual necessity*, so long as the *back lens of the objective* is filled with light. Mr. Poser (formerly manager of Carl Zeiss in London), whose knowledge of the theoretical and practical details of the microscope commanded great respect, used to point out he believed that, whilst agreeing with Mr. Conrady in the importance of filling the back lens of the objective, the best practical position for the condenser to focus in is just *above* the front lens of the objective in use. We have ourselves also, in the past, spent much time making numerous experiments, photographic and otherwise, in connection with this subject, and have come to the conclusion (i) that filling the back lens is *the* important factor to obtain the best definition, and for that reason the microscopist should always look at the back lens of the objective (to see such condition is fulfilled) *before even examining his specimen*; (ii) that seeing the ground glass of the Nernst or other electric lamp, or the flame-edge *actually* in focus is often objectionable, more especially the former, *no injurious effect* is produced in the definition by either raising or lowering the condenser *a small amount* provided the back lens is not emptied in the slightest degree by so doing, and that (iii) we think Mr. Poser is correct when he states more light is obtained by raising the condenser *just* above, rather than by lowering it *just* beneath the position where it forms the image of the illuminant, although theoretically there should really be no difference. If it be desired to pursue the matter further, let the doubting mind consider the following: When the edge of the flame is focussed, is it in reality the *actual* edge that is in use? Presuming it is so, does it make any difference—and if so, what—should the *centre portion* of the wick be focussed instead whilst the edge is still presented to the mirror? Likewise what change in definition follows should the light corresponding to the very furthermost edge of the wick be

employed instead of either of the preceding? It must be allowed there is no change. Seeing this is so, it is obvious a range of focus exists from the front to the back of the wick, which, being granted, shows the truth of what we have said, viz. that a certain "play" of the condenser is admissible—*it being always provided the back lens of the objective is equably* filled with light.[1] It must not be thought in saying this we are backing out of the necessity, so often stated, for always obtaining critical light: we mean nothing of the sort, but merely that recent researches have *broadened out the definition* of which is actually meant by the term "critical illumination" and its method of production.

With respect to the selection of illuminators to suit objectives of different focal length we have already spoken when dealing with condensers as a whole, but it remains to explain the special object of having different focal lengths at all. Putting aside the utility, which is obvious, of having them of the same N.A. as the objective, or anyhow not less, the use of different focal lengths is to regulate the *size* of the image of the illuminant or of the illuminated area of the field of view, as well as the *intensity* of the light. This is of course obvious, for the *larger* the image of the illuminant the *feebler* the intensity of the light. Should, however, the image of the illuminant be too small at any time, the aperture of the condenser being correct, it can be readily broadened by using the auxiliary or bull's-eye condenser between the mirror and the source of light. In doing so it had better be fidgeted about so that its position does not spoil definition, which it may do if incorrectly placed. Care should be taken in doing this that the back lens of the objective is filled *equably* with light; for unless this be the case, the critical state of illumination will be lost, and the consequent array of troubles ensue, just as obtains when an ordinary substage condenser is badly placed.

Most if not all of the ordinary bull's-eye condensers are poor examples of the optician's art, even the best being not superior to the finest lantern condenser. To remedy this, and supply a much-felt want, Mr. Conrady took the trouble to compute,

[1] This bears out what Mr. Nelson has maintained so stoutly for many years, viz. that the back lens must not show *any* portion of its area unequally illuminated by dark spots; if these exist, the adjustment is *not* correct.

and Messrs. Watson & Sons have made, an achromatised aplanat of the very highest quality. We have enjoyed the use of one of these for some time. It can be employed not only as a very perfect bull's-eye, utilising all the useful light from the illuminant, but the optical parts in our arrangement are made to lift out and be placed in the substage, where the aplanat makes a very magnificent low-power condenser of great utility with objectives of suitable focal length.

There are one or two *mountings* for these auxiliary condensers which have proved very useful over and above those ordinarily

Fig. 103.

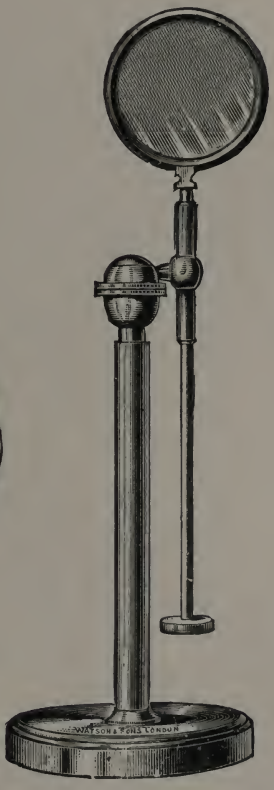


Fig. 104.

met with. One is by Leitz—a firm whose fame has justly increased by leaps and bounds in recent years—which we give in Fig. 103. The lens is large, the mount very steady

and not likely to get out of adjustment, and the price exceedingly moderate. Messrs. Watson & Sons, another firm whose ambition is to turn out articles for the microscopist of all sorts, lenticular and otherwise, as much up-to-date as possible, have some while ago introduced a very portable and convenient ball-and-socket stand of much service. It is illustrated in Fig. 104. We have had one in use for some time as the mounting to the Conrady condenser, and are quite satisfied with its performance.

Messrs. Bausch & Lomb have also introduced a modification

Fig. 105.

which we think, simple as it is, an excellent one. It consists in fitting a counterpoise to the bull's-eye at the other end of the arm which supports it. This is to prevent its dropping by its own weight, and does away with the necessity of clamping-screws to hold it in position; but it requires a little practice to use. It is shown in Fig. 105.

C. Baker has also a pattern with lamp complete; a rack to raise it up and down, and one to move it side to side with the bull's-eye, itself capable of being turned aside when not required, completing a most useful combination. This is indeed a very serviceable arrangement, for the bull's-eye is very quickly brought

into use, and equally quickly turned aside when not required. Like this firm's characteristic attribute, every detail has been carefully thought out—although perhaps the *idea* originated from without—and perfected by the experienced and exceedingly skilful manipulator, both as microscopist and photo-micrographer, the late Mr. Lees Curties, who was the leading spirit in this department of the firm. It is illustrated in Fig. 106. Space will not allow us to illustrate any more of these bull's-eye condensers, but we should mention that other equally good arrangements are made by Messrs. R. & J. Beck, Powell & Lealand, Ross, Swift, and others; although none of them, we believe, claim to be optically equal to the Watson-Conrady aplanat of which we have just spoken.

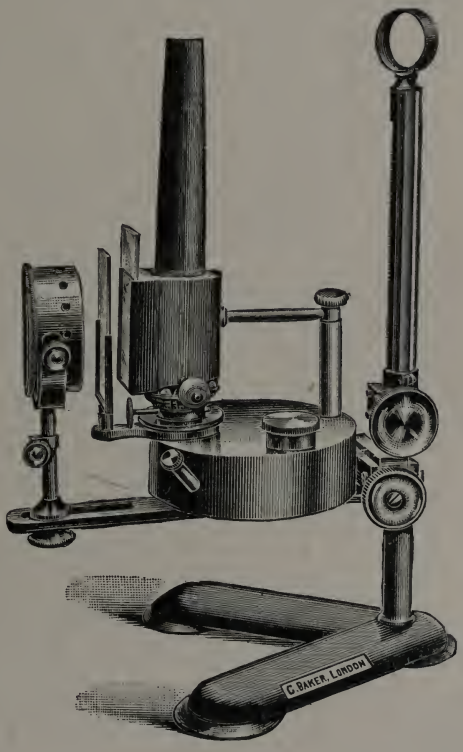


Fig. 106.

The selection of a suitable illuminant depends very much upon the requirements and individuality of the microscopist, for some like a bright light to use, whilst others prefer as soft an illumination as possible. For every-day work, where electric light is not available, the microscopist's ordinary oil lamp, as shown in Fig. 106, is, we suppose, the source of light most frequently met with. It is not always found associated with the extra details as given in the cut, and may be procured in almost any form and at any price. The Welsbach mantle used with coal gas is a favourite with several, but its great heat is a drawback, although its

freedom from smell and dirt and its readiness for use make it a decidedly handy form to employ.

Where electric light is available, the Nernst five-shilling lamp was of great service, and if its filament and furnace had been made in a more durable form, it might have superseded many other electric lamps for general use. Failing this, we strongly recommend an excellent thirty-candle incandescent lamp by Stearn called the Zigzag. In procuring either of these lamps, half of the bulb should be fine *sand-blasted*, as the transparent glass, or even the *glacé* variety of globe, is not suitable for focussing when obtaining critical illumination. Besides the above-mentioned illuminants, there are new ones mentioned in the Addenda to which the reader is referred.

Two more illuminants introduced a few years ago remain to

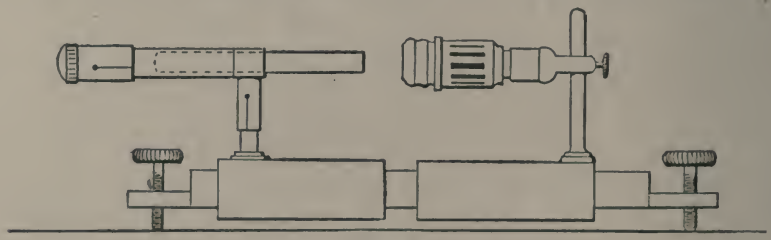


Fig. 107.

be mentioned, although not much used we believe. The first is an adaptation by Mr. J. E. Barnard of the Mercury vapour lamp for the use of the microscopist. Although this is, unfortunately, a very delicate arrangement to handle, and will not bear transit unless very carefully packed (and even then may suffer through very violent shaking), still it is unquestionably an exceedingly fine illuminant and one that may be said to be almost absolutely monochromatic for the apple-green. It can be obtained at Charles Baker's, 244, High Holborn, London.

The other is one that has been brought into notice by Mr. J. W. Gordon. It consists, as shown diagrammatically in Fig. 107, of a Nernst lamp in close proximity with a glass rod of about half an inch thickness, having the end nearest the filament finely ground, whilst the other is lenticular in form so that the rays shall be brought to a focus upon the mirror of the microscope. Approaching the glass to the filament very rapidly and sensibly increases the intensity of the illumination, whilst

removing it further away has of course the opposite effect. Although thought of by Mr. Gordon quite independently, the idea of using a glass rod for this purpose is no novelty, as a lamp based upon this principle was sold by Carl Zeiss some twenty years ago; but Mr. Gordon's adaptation of the idea to an electric light, the neatness of his arrangement of fittings for presenting the rod always at right angles to the filament, and the adjustments for raising or lowering the intensity (which necessitates the rod being made of one of the new forms of heat-resisting glass) place the lamp, as he presents it, upon what we may almost call a novel basis. It certainly is a pleasant illuminant to use and furnishes a very equally distributed light all over the field.

Fig. 108.

If any of these different illuminants are not of sufficient power when oblique light is used with a monochromatic screen, or for employment with high-power dark-ground illumination arranged for viewing bacteria, the miniature arc lamp originally introduced by Leitz, but now made by Watson & Sons under the name of the Argus Lamp, Fig. 108, should be employed. The new Pointolite lamp is recommended also for the same purpose (see Addenda). In the usual form of arc light the two carbons are either in a vertical line one with the other, or else are both inclined at an angle in such a manner that the crater points towards the object to be illuminated or the condenser that has to be filled with light. In this new form, however, the conventional position of the carbons is entirely

changed, for they are placed at *right angles* one to the other, and the positive or upper rod, which is cored, is *thinner* than the negative (instead of thicker as usual), being made the longer of the two, so as to have a life of sufficient duration. It is claimed by the inventors of this system that much more light is obtained by the new position of the carbons as well as by the smallness of the positive element. It is fed by hand, but when desired can have a long arm attached to it so that the microscopist is able to manipulate and adjust the carbons without taking his eye from the instrument; a matter of no small moment as it prevents the glare of the light spoiling the sensitiveness of his retina. The lamp only takes four ampères, and can, in conjunction with a suitable resistance supplied to order, be switched on to the ordinary house system without any trouble whatever, save the possible strengthening of the fuse in the fuse-box for the circuit in use.

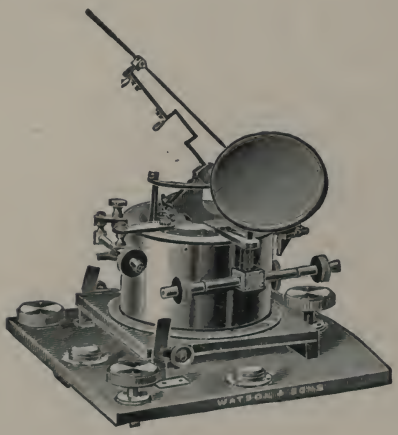


Fig. 109.

A bull's-eye condenser is fixed to the front of the lamp which is easily removed when not required.

Daylight is not desirable as an illuminant for the microscope, owing to its diffuse nature; but sunlight, if utilised in the proper manner, leaves nothing to be desired save the little that it is seen in winter in this country. A difficulty, however, which prevents its more frequent use is that owing to the earth's rotation there is great trouble—in fact, it is impossible with ordinary means—to keep the image of the sun *always* on the mirror of the microscope. To do this effectually so that it will not shift, even upon different parts of the mirror, an instrument has to be used called a Heliostat. This is a very expensive apparatus, and unless it be really a good one is of no use at all. We know of several; but we believe the best, and certainly one of the most convenient, is that manufactured by Messrs. Watson & Sons, according to the instructions of the late Dr.

Johnston Stoney (Fig. 109). This is only to be expected when designed by a man of such eminence, one whose ingenuity as a mechanician was only equalled by his mathematical knowledge in all the subtle theories connected with the optics of the microscope. Full instructions are issued with the instrument, so we need not wait to explain how the arrangement is used; it must suffice, therefore, to say that when once set upon the sun it projects its image upon the mirror of the microscope, where it remains apparently quite stationary. Those using the sun's light for the first time are especially warned *not to place their eye to the ocular at all without using a protective monochromatic screen*—and a deeply stained one too—intervening between the mirror of the heliostat and the mirror of the microscope, or else between the eye and the ocular.

Arrangements for producing Approximate Monochromatic Light

The object of using light of one colour is of a twofold nature: (i) the first being to aid resolution, and the second (ii) to assist in providing greater contrast between different parts of a specimen.

(i) Ordinary so-called white light may be said to consist of all varieties of wave-lengths, say, from $\frac{1}{30000}$ in. (red) to $\frac{1}{60000}$ in. (violet); its mean wave-length in air, however, being usually spoken of as about $\frac{1}{47500}$ of an inch.

Seeing that Abbe's Law for ascertaining the theoretical power of resolution with any objective is to multiply twice the number of waves to the inch of the light used by the N.A. of the objective, it is very obvious that twice the resolution, roughly speaking, is obtained by employing violet light rather than by using red (see p. 156). To be able then to use blue or blue-violet light has long been the desideratum of the microscopist. Hitherto no blue glass could be made that even approached monochromaticity. That sold by Zeiss was a great advance for photography, but left much to be desired for visual work. The comparatively new films made by Kodak Ltd. are exceedingly fine, and are spoken of at some length in the Addenda; but *perfectly pure* monochromatic illumination can only be obtained by the use of the blue light formed by spectroscopic means, concerning which we shall now speak.

This method to which we refer is by employing either a prism or a grating in some form. The firm of Carl Zeiss make a

train of prisms that is attached to the under part of the stage: we hear it well spoken of, but have not tried it ourselves. Mr. Nelson has designed another arrangement, but when using it we have not met with the success we expected. Blue *fluid* filters have also been employed, more especially ammonio-sulphate of copper, in cells measuring about 3 in. in length, 2 in. in breadth, and $\frac{1}{5}$ in. in thickness; but the great difficulty of using any of these fluid arrangements is the large amount of light absorbed thereby. If the Argus 4-ampère lamp (see page 167) be employed, and the solution be concentrated, a fairly

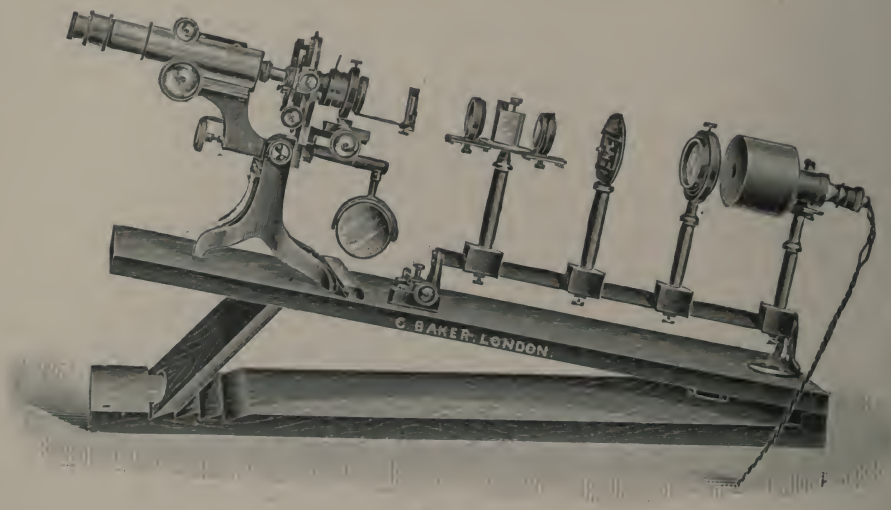


Fig. 110.

favourable light-filter is formed with the ammonio-cupric-sulphate or with the acetate of copper solutions; but neither can be called truly monochromatic, for they nearly always pass red rays so far as we have ever seen.[1] The only means we know of to obtain an absolutely perfect blue light is by the employment of the special arrangement devised by the author, and exhibited at the Royal Microscopical Society several years ago (Fig. 110). Besides details of an ordinary nature—which can be

[1] The two following formulæ are said to furnish good results: (i) Pure copper nitrate 160 grains, chromic acid 14 grains, with water to $7\frac{3}{4}$ ounces; (ii) A saturated solution of copper acetate.

seen in the figure—the machine is constructed to furnish *prismatic* light of any colour by employing one of Thorpe's replicas, which is attached to a glass prism specially designed to keep the blue and green rays parallel on leaving the prism, to the incident beam projected on the replica. By this arrangement, which in its final form is very convenient and handy to use, limelight, or the Argus 4-ampère lamp can be employed, although for many purposes the five-shilling Nernst lamp, as shown in the figure, is amply sufficient (see *Journal Roy. Mic. Soc.*, December, 1902, part vi., p. 727).

The F-line fluid screen by Gifford and the Pot-green glass[1] described by the author in previous editions of this work are still much used, but we think are now eclipsed by the gelatine films made and sold by Kodak Ltd. Greens are to be had in many varieties, and are cheap, only requiring mounting between two lantern-slide cover glasses. The best we think are Nos. 66, 56, and 61, the last being the deepest. None are *truly* monochromatic, all passing a little of the red end of the spectrum; but if No. 61 be combined with No. 44A—a kind of blue—a very perfect green results, but it wants rather a strong illuminant. Blue screens, and how to use them, will be found in the Addenda.

(ii) The second use of screens is to create a greater contrast in different parts of a specimen. Such use is, we are ready to admit, more of service in photomicrography; but it is of much *comfort* especially when employed during a hurried examination through several specimens, for it prevents very materially the exhaustion of the eye. We recommend this detail not only for this purpose, but because using a suitable green screen causes objects stained red, for example, to appear black; hence they are easily distinguishable, although the general illumination is so much softened. The green glass above mentioned may be used for this purpose with specimens stained other colours; but

[1] This particular glass, which is not to be confused with another variety of pot glass called "signal green," is very cheap, and sold by Charles Baker, 244, and by Messrs. Watson & Sons, of 313, High Holborn, E.C., being often called after the author's name. It has an advantage over the fluid screen, namely, that it does not fade with age; moreover, it will not readily break. Different thicknesses are sold, but it is not completely monochromatic.

more especial directions and details are given in the chapter devoted to the use of the microscope in Bacteriology.[1]

Dark-ground Illumination

Dark-ground illumination is used by the microscopist to show up the details of an object in a somewhat remarkable and at the same time somewhat beautiful manner. The idea entertained in the method is that the object shall be lit up by extremely oblique rays *only*, and by no direct ones at all. All manner of curious silvery-looking effects are thereby introduced, which in popular demonstrations of the instrument are often greatly admired, although as a source of *investigation*, however, not so much is gained thereby as might be expected, save perhaps in learning the general contour and actual configuration of living bacteria as explained later on.

The usual method adopted *with dry objectives of low magnification* is twofold, either by employing the Spot Lens (or its equivalent, a "wheel stop," placed beneath the ordinary substage condenser) or by the Paraboloid.

The Spot Lens is merely a condenser with a piece of black paper more or less permanently attached to its lower lens in the centre. In some cases, instead of using paper, the glass is itself ground rough and painted black in the same situation. The disadvantage of this method is that the diameter of the black disc only *really* suits the individual objective for which it was made, and it is expensive to have several condensers, each being ground and painted to suit a battery of objectives; and it is equally inconvenient to have to cut individual pieces of black paper to affix to the condenser for every combination used in the microscope. To meet this trouble stops are often used that are made like "wheels" with only three very thin spokes. The rim is also thin, but the "box" of the wheel is made in different sizes. By having several "wheels" to suit the numerical aperture of different objectives, one can usually be found that, placed in the carrier beneath the condenser, stops off the direct light, whilst still allowing the oblique rays to pass. The peculiarity of this illumination depends upon this very fact, and the

[1] Mr. Rheinberg, by the use of his original method of arranging differential coloured illumination, described a little later, thinks contrast effects can be produced almost equal, if not quite so, to that by the ordinary method.

characteristic effect produced thereby is that, whilst the object is itself brilliantly illuminated, the background remains perfectly dark. A certain amount of raising or lowering of the condenser and wheel is necessary, and the substage iris may have to be closed[1] a little before these conditions are fulfilled; but it is more of importance, if the *very* best effect be desired, that the "box" of the wheel-stop shall be exactly of the correct size, which in point of fact varies according both to the numerical aperture of the objective *as well as* the focal length of the condenser in use. Generally speaking, a wheel-stop is supplied by the optician the correct diameter for employment with any given objective when used with any particular condenser, and consequently but little information is given in any text-book how to make the necessary calculation. As, however, it is convenient for the microscopist to be able to do this for himself for any combination of objective and condenser, and also as it will be found of service when utilising the Traviss expanding stop—about to be explained—we furnish the rule, which is extremely simple. First, with respect to the objective in use, the N.A. to be "stopped out" is usually reckoned at about 10 per cent. *larger* than that of the combination itself; hence, for example, if using an inch objective N.A. ·30 the amount to be occluded would be N.A. ·33. The diameter of the stop itself—the "box" of the wheel—is then found by *multiplying twice the N.A. to be stopped out, by the equivalent focal length of the condenser in use.*[2]

Owing to the trouble in *procuring* wheel-stops of exactly the correct dimension, an ingenious idea suggested itself to Mr. Traviss that he should devise an *expanding* stop, one in fact that could be increased or diminished at the will of the operator. It can be used with any low-power combination, and really consists of the reverse of an iris diaphragm. With an ordinary iris, as the handle is turned, the aperture becomes less and less,

[1] Carl Zeiss provides a diaphragm of given diameter to be dropped *over* the low-power objective when it is to be used for dark-ground illumination. This avoids the necessity of altering the substage iris. If a "Davis Diaphragm" be used it may have to be closed a little to perfect the definition and the blackness of the background. See Accessory Apparatus.

[2] It should be understood that this rule assumes the stop is placed close to the posterior focal plane of the condenser. See Appendix concerning Position of the Planes.

but in Mr. Traviss's invention it *opens the leaves instead*, thereby increasing the diameter of the stop (Fig. 111).

There are two forms, as shown in the accompanying figure. One is mounted on a stem to slip *into* the under part of the sleeve holding the condenser if such be long enough, whilst the other is arranged to be used in the ordinary position occupied by the "wheel," viz. immediately beneath the condenser. The little handle shown in the figure in both forms serves to expand the leaves and so increase the diameter of the stop.

In using this expanding stop a few hints may be of service. Seeing that in most cases with dark-ground illumination a

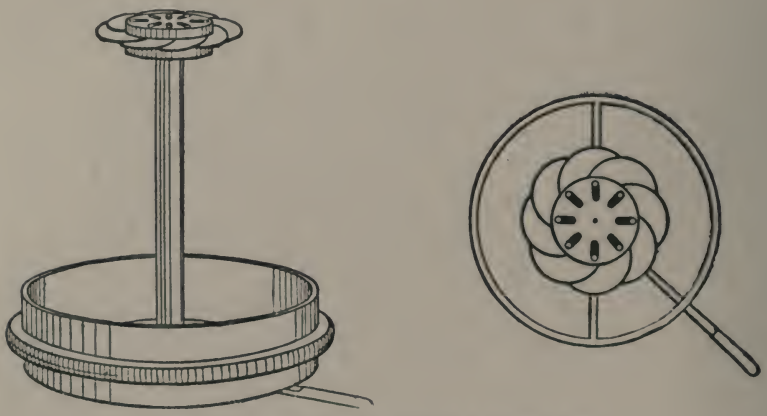


Fig. 111.—Mr. Traviss's Expanding Central Stop.

certain portion of the periphery of the objective in use has generally to be cut off by the substage iris diaphragm or by the Davis shutter if that be used instead, to make the background sufficiently dark, it is obvious the less this is necessary the less the reduction in the resolving power of the combination. Hence we have found with Mr. Traviss's stop it is a good plan to leave the iris substage diaphragm or the Davis shutter *wide open* to commence with, and to extend the leaves of the expanding stop as far as necessary according to the rule for ascertaining the correct diameter. Moving now the stop and the condenser in use with it a little upwards towards the specimen, or downwards away from it, the position is sought that produces the best illumination of the object coincident with the darkest

ground procurable, *only altering the diameter of the expanding stop if required.* When this is effected the Davis shutter or the iris substage diaphragm is slowly closed to bring about the perfection of blackness desirable in the background *with the least possible amount of closure.* It will be easily understood, by arranging the details in this manner and in this order, the *effective* aperture of the objective is reduced the smallest amount, and hence that the definition of the object suffers to the least possible degree.

Used with low powers we confidently recommend this useful piece of apparatus which was made in pre-war times to expand to more than an inch and close to about three-eights, a triumph in ingenuity of construction. Care is required to be sure it fits the understage sleeve accurately, unless the whole ring will move round when the handle is turned instead of only the leaves. The ring is cut to open or close for this purpose.

It would appear as if we were guilty of a decided omission if, before we concluded our remarks upon this kind of illumination so far as it relates to its use with low powers, we neglected to mention a curious and beautiful modification discovered and elaborated by Mr. Julius Rheinberg, F.R.M.S., and called by him "multiple or differential colour illumination," more especially as it seems to have escaped the attention of so many microscopists. It appears to have suggested itself to him quite by accident after placing a piece of coloured glass between the illuminant and the mirror on one particular occasion when using dark-ground illumination. He noticed then that "the *object* was illuminated with the colour of the glass much more so than the background." Following up this little discovery, much time was spent in devising and perfecting methods whereby colour differentiation between an object and its background, or between parts of the same object, could be attained by optical means, resulting in three quite different methods being found: one of them—named the refraction method—applicable chiefly to lower-power work; the other two—named the diffraction and composition methods respectively—being chiefly for high-power work.

As the refraction method, suitable for use with objectives like an inch, is the easiest to apply, and shows what results may be

attained, perhaps even better than by either of the others, we shall confine our subsequent remarks to this one; but the reader who is anxious to further follow up this interesting subject will do well to consult the original papers given in the footnote.[1]

The principle involved in the refraction method is nothing but a modification of ordinary dark-ground illumination, and consists in using, as a central stop to the condenser, a pronounced, clear, and transparent colour, say *blue*, whilst the remaining portion of the illuminator is covered with some other colour, as, say, a well-marked *red*. For example, a disc of red gelatine (such as that used with crackers) is cut to fit the ring usually found in some form beneath the substage condenser of most modern microscopes. A hole is punched in the centre of this equal to about one-third of its diameter, in which is fitted a blue interior of the same material. The "fit" need not be so exact as it might be imagined is necessary, for what may be called a loose one does perfectly well. This bi-coloured disc is then dropped into the ring beneath the condenser, and the adjustments made similar to those that obtain when ordinary dark-ground illumination is employed.

Presuming an inch objective is in use, the result of this method of illumination is very astonishing, especially when objects such as Polycystina, Diatoms, or even living organisms like Rotifers are placed on the stage, for the background is blue, whilst the little objects are coloured brilliantly red.

Combinations of colours having a marked contrast produce the best discs, but it should always be recollected the colour of the central portion must be *less bright* than that of the peripheral,

[1] "On an Addition to the Methods of Microscopical Research, by a New Way of Optically Producing Colour Contrast between an Object and its Background, or between Definite Parts of the Object itself," *Journal Royal Microscopical Society*, 1896, pp. 373-88.

"Note on Coloured Illumination," *Journal Quekett Microscopical Club*, 1897, pp. 346-7.

"Note on a New Modification of Double Colour Illumination," *Journal Quekett Microscopical Club*, 1897, p. 438.

"Notes on Colour Illumination, with Special Reference to the Choice of Suitable Colours," *Journal Royal Microscopical Society*, 1899, pp. 142-6.

"Multiple Colour Illumination," *Illustrated Annual of Microscopy*, 1899, pp. 44-50. Percy Lund, Humphries & Co., London.

On the shape of different screens for obtaining the best effects with Multiple Colour Illumination. See Addenda.

a point often overlooked in the discs sold by opticians for this process of illumination; an unfortunate circumstance, for the best results cannot be obtained under these circumstances.

Very beautiful effects also result with certain specimens by using a coloured centre *only*, leaving the rest of the disc white; but a positively remarkable arrangement is one where the coloured pieces are shaped in such a manner that when the disc is used with a piece of silk muslin on the stage, the *transverse* fibres are illuminated by one colour, and the *vertical* ones by another! Mr. Rheinberg, in his contribution to *The Illustrated Annual of Microscopy* (1900), described how this particular disc was made, but as the publication is now out of print, the particulars are inserted in the Addenda.

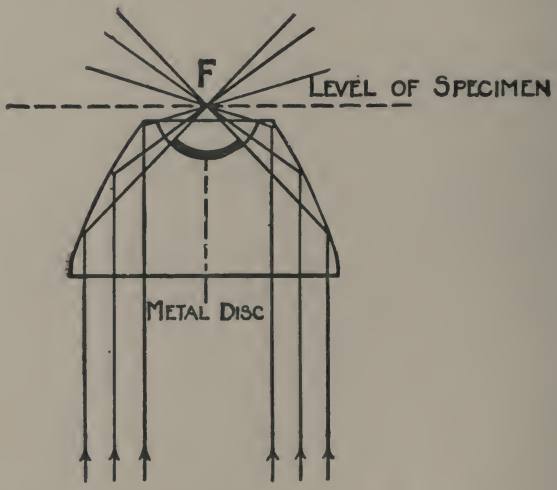
It is scarcely necessary to offer an explanation of these different phenomena, for any one who has mastered the simple philosophy of dark-ground illumination will understand them quite readily. It is obvious in the case mentioned, red outside and blue inside, that the blue central disc, taking the place of the usual black one, furnishes the colour of the *background*; whilst the oblique rays that usually illuminate the object with white light are *coloured red* (in this instance) by their transmission through the outlying red portion of the gelatinous disc. The extension of the application of this method of illumination in the many directions explained in the original articles by Mr. Rheinberg is entirely due to his painstaking and carefully conducted experiments, and we must say we do not think he has received the credit due to him for the amount of patient work necessarily involved in such a class of research.

We ought to mention that it has been argued by some microscopists that this method of illumination merely furnishes spectacular effects, and does not offer additional information as to the formation of structures or organisms examined by its means over and above those obtained by the use of ordinary dark-ground illumination; but we must say the direct contrast presented to the eye by the object in one colour *lying on a background of another* furnishes a means of differentiating that is of great service when examining the general contour of an object, and it is hardly questionable that the transition in hue from one colour to a *strongly contrasting one* assists in a more vivid realisation of the disposition and relative thickness of different parts of an object than results from examining the

"studies in black and white" as obtained with ordinary dark-ground illumination. We might mention incidentally, and in conclusion, Mr. Rheinberg considers he gets in many cases, by the use of this method of illumination, the same benefits, or what appear to be the same, as those arising from the use of "monochromatic" screens.

(ii) **The Paraboloid.**

This seems to have been something of a mixed invention of Messrs. Shadbolt and Wenham, although each worked on



WENHAM'S PARABOLOID SHOWING PATH OF RAYS & APPROXIMATE POSITION OF SPECIMEN

Fig. 112.

somewhat separate lines at first. It consists, as shown in Fig. 112, which is drawn in section, of a piece of glass shaped as a paraboloid, but flat at its larger or lower end, and deeply curved at its upper extremity. In the centre of this is placed a piece of metal, curved or flat, but anyhow so as to obstruct the direct rays from the illuminant. It needs no explanation to follow the path of the rays, which on consulting the figure are seen to be reflected off the parabolic surface into the glass again to quit it at the deeply curved upper extremity. The object of this deep cutting away is that the rays may leave "normal to the surface," and so not be broken up into coloured

ones. They impinge, as will be seen, upon the object placed in the focus F, and scatter afterwards somewhat irregularly. A certain few, caught up by the low-power objective, are carried to the eyepiece. For convenience in use, the little central piece of metal is fixed to a stem, which although not shown in the illustration really passes right through the axis of the paraboloid, ending in a little knob beneath. An up-and-down movement can by this means be applied to the metal disc so as to arrange the darkened portion of the field in accordance with the numerical aperture of the objective, and the diameter of the object and the general requirements of the case.

It is obvious in the employment of this arrangement the ordinary condenser is removed, for the paraboloid is usually made to be placed in the sleeve in its stead.

Reference should here be made to a method for obtaining dark-ground illumination which has been suggested by Mr. J. W. Gordon. The idea was, so far as we understand it, that the object should be lighted by a condenser in the ordinary way and that the direct light should be stopped out by a central stop either behind the objective or, preferably, above the eyepiece in the so-called Ramsden circle.[1] Whilst there can be no doubt that the appearance connected with dark-ground illumination, viz. that of the object standing out bright against a dark ground, can be perfectly realised in this manner, the proceeding would seem to be one dangerously inviting the formation of false images. Dark-ground illumination at its best is apt to yield deceptive images, as has been repeatedly proved at the Royal Microscopical Society by instructive and convincing experiments, shown both by Mr. A. E. Conrady and by Mr. J. Rheinberg. It is highly probable then the possibilities in this direction must be enormously multiplied when part of the full aperture of the *objective* is blocked out by a stop in the Ramsden circle; but it is quite certain that such placed behind the objective itself is a most fruitful source of false effects. Microscopists cannot be too often reminded that the most startling "nightmares" included in the famous experiment with

[1] The Ramsden circle is fully explained on page 118, where it is shown the diameter is equal to—

$$\frac{500 \text{ mm.} \times \text{N.A.}}{\text{Total Magnification}}$$

Abbe's diffraction-plate were obtained by the use of such stops, and that images so utterly false *cannot*, as a strict matter of fact, be obtained *by any means whatever* if the normal round aperture of the objective be left undisturbed. The extraordinary appearance of blood corpuscles having "sculptured surfaces," recorded by Mr. Gordon by his method, it would make one think belong undoubtedly to this order of "invited phenomena." It is obvious then, with but little further consideration, that the arrangement he suggests must be used with the greatest possible caution *as a means of research*, and no result obtained should be depended upon unless amply confirmed by ordinary and more legitimate methods of observation.

This concludes the explanation how to obtain dark-ground illumination with low powers; all that hitherto appealed to microscopists in general. Lately, however, a new departure has taken place, for several opticians have computed and constructed special forms of oil-illuminators of large numerical aperture that can be very profitably employed with high powers, such as a dry sixth or an immersion twelfth. Seeing that the utility of this extension of the use of the method in question may not be immediately apparent, it will be well to state that up to the present the microscope has not furnished satisfactory results when employed upon small transparent objects, whose refractive index has not differed to any sensible amount from the fluid in which they are immersed, such for example as living bacteria in water or other similar fluid. It is true those that were skilled have —by closing the substage diaphragm a sufficient amount—been able to see images (largely mixed up with diffraction phenomena and consequently of a doubtful nature) sufficiently well perhaps to identify with more or less certainty one species, and to distinguish it from another; still it has been generally acknowledged even by these trained observers that the method and the observations left very much to be desired. Now it is by this extension of dark-ground illumination to the use of high powers it is hoped that, anyhow, most of the difficulty will be overcome, for by its use not only can these little objects be seen well enough to distinguish the organisms with ciliæ from the non-motile, and the naturally rapid and darting movements peculiar to the one type, from the torpid and perhaps irregular motion of other forms; but, what is of far more importance still,

is that these little bacteria can now be studied in their *natural and living state*, rather than by examining specimens after staining and mounting, a process that is acknowledged to produce differing results according to the stain used, the length of time it was employed, and the particular method adopted.

It was thought that this new departure, first suggested by Herr Reichert in a lecture before the Congress of Naturalists held at Stuttgart, November 1906, would have a great future before it; but subsequent experiences have not shown this to be the case. Details are given, however, to obtain the best results.

There is no difference in the philosophy of this extension to high powers over and above that which obtains when dark-ground illumination is employed with low powers, for the objects have to be illuminated as before, solely by the extremely oblique rays (those passing direct to the object up to a limit of N.A. 1·0 being cut off by a stop suitably placed below the condenser); hence the difficulties to which reference has been made are not due to change of the principle, but to change of the means adopted to bring about the desired results.

Before proceeding, the questions may very naturally be asked: Why cannot the ordinary oil-condenser be used for the purpose if provided with a stop of suitable size, and placed in the usual position? Why have opticians made special devices for the purpose? To this we reply that it is quite possible when using a sixth, if its aperture does not exceed about N.A. ·7, to obtain a fairly good dark-ground effect in the ordinary way with the ordinary means, but that it has been found, when a higher aperture is required, it is impossible to realise a good dark-ground effect all over the field. Moreover, that it is not easy either to find how to fix the stop in exactly the correct position, which must be close to, if not exactly at, the posterior focal plane of the condenser, or to ascertain what its exact diameter should be so that it cuts off all rays below N.A. 1·0. Still further, owing to the great sensitiveness of this type of illumination to colour effects, it has been proved that far better results, images of a far purer character, are obtained when the necessary bending of the rays to a focus by the condenser is brought about by reflection rather than by refraction as ordinarily obtains with the usual type of illuminator.

Several of these new forms of condensers have been placed

on the market, notably by Leitz, Zeiss, Beck, Reichert, and others, two or three of which it must suffice to describe. In each case the arrangements are such, that the rays below N.A. 1·0 are cut off by a stop suitably placed beneath the condenser, whilst those above that aperture are united by reflection instead of by refraction. Another question may here be asked, If all the rays below N.A. 1·0 are cut off by the stop, how can the bacteria be seen with a dry objective, seeing that its aperture is never above ·95 or thereabouts? In replying to this it will be of interest to follow the course of these extremely oblique pencils, and to learn how the seeing is effected under these apparently paradoxical circumstances. Leaving the upper surface of the condenser they first traverse the oil connecting it with the under side of the slip. Quitting this fluid, and having entered the slip itself, they pass through it to gain admission into the water containing the bacteria. Here they strike these little organisms and illuminate them so brilliantly as to make them appear as self-luminous bodies, by which means they are enabled to be seen by the dry objective when used in the ordinary manner. Tracing the path of the rays which happen not to fall upon any bacteria, we find them passing through the water and entering the cover-glass. As they arrive at its upper surface at a very acute angle—less in point of fact than the critical angle of the glass from which the cover was made—and being unable in consequence to enter the air, they suffer total reflection back again into the water. The direct consequence of this is, that with a dry lens the only pencils gaining admission into the objective are those coming from the bacteria, whilst the effect is, that these bright organisms appear projected on a perfectly dark background. When, however, an immersion twelfth is employed, the contact of the oil permits these rays in question to quit the cover-glass, and so they now enter the front lens of the combination, which means the background is no longer dark. In consequence of this, to make it so, it becomes necessary to add a diaphragm or a set of diaphragms to the immersion system so that the desired effect may be thus obtained. Owing to this necessity of cutting off these rays in question, some opticians are of opinion that the only benefit that accrues from the use of an immersion twelfth, is that due to its shorter focal length, namely that it magnifies more than the

dry sixth, which they aver can just as well be procured by using a higher ocular upon the dry system. In reply to this we think we ought to point out there are certainly other advantages of using the immersion combination referred to, which it is possible have escaped their attention for the moment. The first is that by its use the necessity for using a cover-glass of specified thickness is avoided, for reasons too obvious to mention; the second is that the image is undoubtedly brighter owing to the absence of all reflections from the surface of the cover-glass, and at that of the front lens of the dry system; whilst the last rests upon the fact that, although it is true the aperture of the immersion combination does require cutting down to obtain a dark-ground effect, still, notwithstanding this limitation, the aperture remaining is really greater than that of the dry lens, and hence that resolution is in consequence certainly increased thereby.

The condenser made by Leitz in pre-war times was very good (Fig. 113), the optical parts being mounted in a centring device which was of the greatest service in the final adjustments. It will be readily seen by the study of the figure that the rays are twice reflected before coming to a focus at P. As this distance is a fixed quantity—for up-and-down movements of the condenser are not desirable—the firm direct that a slip of 1 mm. should be employed if the best results are desired.

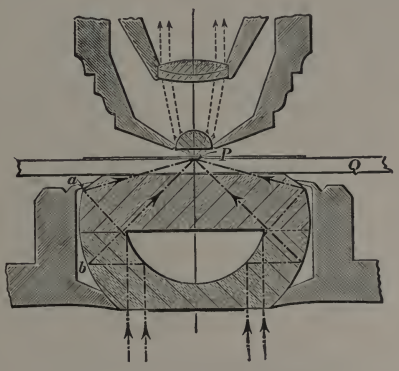


Fig. 113.—Leitz's Condenser.

The pattern sold by Carl Zeiss in pre-war times gave very excellent results, but was of entirely different design. They considered that the paraboloid offered very considerable opportunity for the elimination of both spherical as well as chromatic aberrations. On examining Fig. 114, it will be quickly seen that the bending of the pencils is here again effected by reflection rather than by refraction. Instead of mounting their illuminator in a self-centring jacket, they recommend the use of an arrangement of the same kind, but applied to the objective. To this we

reluctantly demur, as the employment of such, while it puts the condenser and the objective into alignment, it is true, is apt to throw the ocular out of the axis at the same time, unless most judiciously employed. So perfectly made were the goods of this

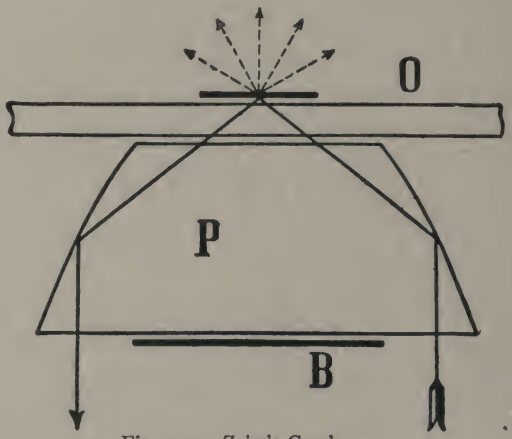


Fig. 114.—Zeiss's Condenser.

firm that we must allow there was no need of any centring device at all when we placed this condenser on our stand; but the point is, when it is used on a microscope of another make and has to be perhaps affixed thereto by an adapter of some sort, it is then that the want of a self-contained arrangement might be very much felt.

The thickness of slip recommended for this illuminator lies between 1·3 and 1·4 mm.

The form of condenser (Fig. 115) sold by Messrs. R. & J. Beck, of Cornhill, consists of a reflecting paraboloid similar in that respect to the last-mentioned illuminator by Zeiss; but it is modified with respect to certain details that we mention, which the makers claim facilitate its use.

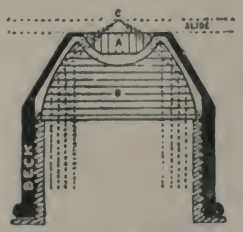


Fig. 115.—Beck's Condenser.

The upper portion of the apparatus is in the form of a lens A (which focusses at C), the upper surface of which is placed in immersion contact with the under surface of the slip, the curved side, concentric with the focus C, being truncated to such an extent as to stop all light from

reaching the object which is of less obliquity than N.A. 1·0. By moving the paraboloid B up or down by means of a milled ring (which rotates the sleeve in which the paraboloid is held) the light is accurately focussed so as to obtain the maximum brilliancy. This piece of apparatus slips into the ordinary adjustable sleeve of the substage and so a self-centring arrangement is not necessary. The slip should be about the same thickness as for Zeiss's arrangement. Mr. Nelson has also devised a parabolic condenser which is well spoken of.

As this type of illumination is particularly prone to show up any dirt or foreign matter in a most disturbing way, great care should be exercised in thoroughly cleaning both slip and cover with alcohol before use, and moreover the microscopist should not forget to gently blow on both just immediately before bringing them into use, so as to remove any small particles of dust which may have fallen thereon after their special cleaning.

A very strong illuminant is required, and we do not hesitate to recommend, after some continual use, the little arc-light originally brought out by Leitz and now made by Watson & Sons, being called The Argus (page 167). It is extremely useful and convenient, for it can be attached to the ordinary house supply, a suitable resistance being supplied according to the voltage of the installation. Or the new Pointolite lamp (see Addenda) is excellent. It can be obtained of different candle-power, and is economical in current. The Zigzag 32 candle-power lamp, made by Stearn, is also of service. It is usually stocked by Messrs. Page & Miles, Electricians, Brighton, and is almost exclusively employed by the Author for ordinary purposes for the microscope. If electric current be not available, a Welsbach incandescent gas burner may be very profitably employed; but the microscopist had better not attempt to use the ordinary oil lamp, as its light is not nearly strong enough to furnish good results.

Whatever the illuminant in use, a bull's-eye condenser is necessary to focus the rays upon the plane side of the mirror. For this purpose Zeiss recommends a glass flask of large size filled with water; it has the advantage of being cheap, but is certainly not handy in point of size; whilst Messrs. Watson & Sons attach a bull's-eye to the little arc lamp, lamp and lens being all in one fitting.

Hints to those who use Dark-ground Illumination with High Powers

To obtain the fullest advantage of this form of dark-ground illumination it is necessary, as we have already mentioned, that the microscopist shall employ a slip of the special thickness mentioned by the manufacturer of the system, for, if this be grossly neglected, it may be found impossible to obtain a field of equal blackness coincident with a brilliantly illuminated object.

The layer of fluid must in all cases be extremely thin, for, if too thick, the background may appear nothing but a light grey instead of a rich black. Should this appearance be presented, and no shifting of the mirror improve the blackness, it is a good plan to gently press the cover-glass, and at the same time apply a piece of blotting-paper to the point of union of the cover-glass with the slip, when it will soak up the expressed fluid and prevent it being again drawn up beneath the cover, as it is very prone to be unless removed by this means, or by using a soft piece of cambric or other material of a similar character. It may be mentioned, if the cover adheres to an immersion objective when such is in use, that it always shows the layer of fluid is too thick.

The light should be focussed on the plane side of the mirror, but the cone of illumination should not (in our experience) be greater than the diameter of a sixpence or a shilling. The mirror may lie in the cone of light from the condensing lens either before the rays cross at the focus, or afterwards, but in general the former position is to be preferred.

If a sixth be used (an apochromat furnishes the best results) it does not require any diaphragm added to its construction, but if a twelfth be employed, such is always necessary. Leitz used to sell a *special mount* to which the nickel portion of his semi-apochromatic twelfth, when removed from its own mount, can readily be fixed. It contains a kind of funnel-shaped diaphragm which limits the aperture of the combination the correct amount; whilst the apochromatic 2 mm. is supplied with a limiting funnel-shaped diaphragm which is screwed into the free end of the combination when used for the purpose. (It should be mentioned that the objective must be sent to the works to be fitted.) Carl Zeiss did not recommend the use of homogeneous systems.

After setting up and attending to the details mentioned (not omitting to oil the slip to the condenser), it is best to first use the apochromatic sixth with as low power compensating ocular as possible, and to focus the bacteria carefully. If the field is all bright instead of black, as may often occur at starting, the mirror should be manipulated in all directions until the objects are very brightly illuminated, whilst the background is black.[1] If this seems impossible to obtain, such difficulty may arise as before hinted, from the layer of fluid being too thick. If no improvement occurs after making it thinner, notwithstanding all positions of the mirror being tried, perhaps the cone of light on the plane side of the mirror may be too large. Presuming no improvement follows making it smaller, it is well to try placing the cone of light slightly to one side of the mirror instead of exactly in its centre. When these points have been carefully attended to it will usually be found that the desired effect is produced. The low-power eyepiece should now be removed and a × 12 substituted. All the necessary magnification required for most objects will usually now be obtained; but, should a twelfth be required, it must be arranged for the purpose, as previously explained.

The microscopist who employs this method of examination for the first time, and who has not had his attention previously directed to the examination of very minute particles floating in a fluid medium—we speak of objects not less than, say, about a five-thousandth of an inch—may easily mistake the peculiar agitation of such that is caused by what is called pedesis, or Brownian movement, for a real motion on the part of the objects themselves. With a little experience the two can be readily distinguished; but if the observer be unacquainted with this peculiar form of motion let him rub a little gamboge with water, and, having boiled the same for sufficient time (to remove the possibility of living bacteria), place a minute quantity beneath a cover-glass and look at the specimen under a twelfth with direct light in the ordinary manner. On examination the peculiar dancing pedetic motion (discovered by Dr. Brown so far back as 1827) can readily be seen, and when once fully

[1] It should be mentioned that some microscopists find better results are obtained when looking for ciliæ, if the background be not jet-black, but rather of a deep grey.

appreciated, cannot be easily confused or mistaken for any proper motion on the part of the little bacteria themselves.

There is another appearance of a striking character—we may call it a wild rushing to and fro of the fluid containing the bacteria—which may very often be seen, especially when the slide is first made. It is not due, it is generally believed, to either of the previously mentioned movements, but rather to the process of equalising the pressures in various parts of the slide, caused very probably by the unequal evaporation consequent upon the irregular distribution of the heat rays in this particular form of illumination.

There is yet one more trouble which may annoy the observer. We refer to the possibility that each of the little organisms or particles may appear to have wings of light, and that larger pieces of foreign matter present may show diffraction bands, both wings and bands all pointing in one direction. The appearance is most disturbing. Both troubles arise from the axis of the condenser not being in alignment with that of the objective. The defect is a troublesome one and often occurs on commencement, for this form of condenser is not so easily centred as one of the ordinary type. If the illuminator be supplied with its own centring apparatus—as obtains with the arrangement by Leitz—the little screws should be turned until the wings disappear; but to effect a radical removal of the trouble it may be necessary to lift out the ocular and to look at the back lens of the objective. This should appear to be *equally illuminated all round the central black stop*; but to make sure of such being the case the eye should be held at some little distance from the end of the draw-tube and be kept rigidly fixed whilst looking down the axis of the instrument, for, if the head be allowed to keep moving about, it is not possible to judge correctly as to the distribution of the light. Supposing this be found to be unequal, the little adjusting screws of the condenser must be manipulated until the ring of light around the central stop appears to be uniform in brilliancy. When the condenser is not provided with a centring device, an arrangement of the same kind must be attached to the objective, and its screws used for a similar object; but, as we have already said, this is a plan we do not advocate, for if the condenser be very much out of centre, and the objective in consequence has to

be moved a very sensible amount, it is apt to throw out the alignment of the ocular, which perhaps may spoil the refinement of the definition.

Before closing this chapter, reference must be made to a form of dark-ground illumination introduced by Dr. Siedentopf of Jena, and called by him "ultra-microscopical." The great expectations, however, caused by its attractive title, we do not think have been realised in subsequent experience. See Chapter XVI.

In all the previously described methods of dark-ground illumination by condensers of one sort or another, difficulties arise when there are a great number of particles in the object distributed in a relatively considerable thickness, so that only a small fraction of the total thickness is in focus; for in this case, all the other particles *not* in focus being also brilliantly lighted, their diffused images spoil the dark background, making it grey instead.

The method advocated by Dr. Siedentopf overcomes this by lighting the object from the side—through the polished edge of the slip; for, the source of light being reduced to a very small width by an adjustable slit, only a most minute thickness of the object may be illuminated; in fact, by suitable adjustment of this slit and careful focussing of the lateral condenser, the observer may so arrange things that only those particles are illuminated which are in sufficiently sharp focus with the objective and magnification in use. By lighting the adjustable slit with very powerful light (arc-lamp) the illumination may be made so intense as to make extremely minute particles sufficiently luminous; hence the name of "ultra-microscopical particles" has been applied to the objects rendered visible by this method. Needless to say, this does not mean that the accepted limit of resolution had been rendered obsolete by this method; for, although extremely minute particles are rendered visible, they are only shown as shapeless spurious discs of the size prescribed by the undulatory theory. The real shape and the real size of the objects are absolutely hidden and can only be inferred—in some cases—by indirect methods. In fact the visibility of small particles under this method of illumination is a precise analogon to the visibility of the stars, the real diameter of which is a minute fraction (probably less than $\frac{1}{10000}$) of the limit of resolution of the eye or telescope which easily sees or shows them. See "Theories of Microscopical Vision," Chapter XVI.

By the courtesy of Messrs. Carl Zeiss we are enabled to show in Fig. 116 a diagram illustrating this interesting method of

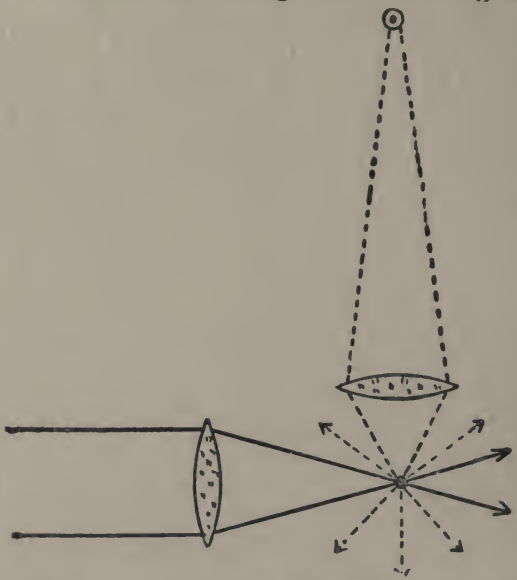


Fig. 116.—Diffraction of Light with an Ultra-microscopic Particle.

Fig. 117.—Set of Appliances for the Investigation of Ultra-microscopic Particles in Fluids, as proposed by Siedentopf and Zsigmondy.

illumination, and in Fig. 117 a complete set of apparatus, including a powerful arc lamp, suitable for the observation of "ultra-microscopic" particles in fluids.

Illumination in General and Oblique Light in Particular

Experiments and mathematical considerations have gradually led microscopists in recent years—notably Mr. Nelson amongst the number—to point out very forcibly the dangers that may occur in using *narrow* illuminating cones ; false and true ghosts, intercostal markings in the image of a fly's eye (!), and many complex and false images with the coarser diatoms become visible, all of which are impossible to be obtained with a wide one. These have been actually photographed. Yet we are at once, for the sake of pure justice in dealing with the question, bound to add, on the other hand, that the late Professor Abbe, so justly esteemed and respected by all microscopists, discredited the whole matter. Indeed, he *actually recommended the use of small cones*, saying that "the resulting image produced by means of a *broad* illuminating beam is always a mixture of a multitude of partial images which are more or less different and dissimilar from the object itself," and moreover that he did not see there was any ground for believing "that the mixture should come nearer to a strictly correct projection of the object . . . than the image which is projected by a narrow axial illuminating pencil."

The situation then is exceedingly difficult to deal with ; for, when the result of the direct *experiment*, conducted with all the refinement and skill of a master hand like that of Mr. Nelson, coupled with a full scientific appreciation of the situation, seems to point *absolutely* and *directly* in the *opposite direction* to the teaching of a mathematical expert and philosopher such as the late Professor Abbe undoubtedly was, one who has never been surpassed, if ever equalled, in acuteness of thought coupled with resourcefulness of investigation in all matters concerning the microscope—we repeat, when these opinions are positively at variance, the onlooker is compelled, from sheer inability, to wait and consider. We are bound to confess, however, after several years of attention to this difficult and far-reaching problem, the weight of evidence in our opinion, taken for what it is worth, certainly rests in favour of Mr. Nelson's view, and we venture to suggest that, perhaps the data upon which the learned Professor built his theoretical considerations may not have included a sufficient "weight" to the teachings of actual experiment ; and hence that, although the theory deduced was undoubtedly correct,

the data from which it was made were insufficiently extensive. For example, bacteriologists seem mostly agreed that the bacillus tuberculosis is probably not an organism likely to have a capsule under ordinary conditions, and yet with a narrow cone, whether the specimen be stained or unstained, a very pronounced encircling capsule, as bright and clear as possible to the eye, appears in *every case*; yet, as the cone is steadily and slowly increased, so does this mysterious capsule disappear! Other illustrations might be given, but we refrain from mentioning many, though we feel obliged to furnish one more peculiar effect of using too small a cone. This is with the common object, the proboscis of the blow-fly. So treated, the exceeding small hairs which lie upon the space intervening between the two lobes actually appear *double*, especially at their tips; whereas with a cone even of *moderate* angle such indication is entirely absent. The question then arises, How far can we reduce the aperture of the substage diaphragm without the risk of introducing false images? To this we reply that, speaking in general, it must not be curtailed to a greater extent than a cutting off of the outer third of the back lens of any objective as seen by looking down the tube of the instrument, the ocular having been removed.

From what has just been said, it may seem exceedingly strange that we should now advance to the *improvement* effected by the use of oblique light in the definition and resolution of an object, especially so, seeing that such is produced by a very *large* curtailment of the back lens of the condenser by the iris diaphragm! It will be necessary, therefore, to show the difference between a narrow cone *per se*, and the narrow beam necessary to produce oblique light.

Before actually proceeding to consider this interesting subject, it is necessary to qualify first what is meant by the distinctive term "oblique light." A mistaken notion has often been entertained that by the use of this class of illumination was meant the employment of a beam of light sent by some means into the full aperture of the condenser *obliquely*. This is not exactly true. Whilst allowing that the mirror if obliquely placed does affect the image—we refer to this when dealing with the testing of high-power objectives in conjunction with the use of the Abbe test-plate—to a certain degree, still this is not what is technically meant when we speak of oblique light.

When using the term in its restrictive sense, we mean the stopping out of the greater portion of the condenser by a diaphragm of say a quarter or eighth moon-shaped design, placed in such a manner beneath the illuminator that the light reflected by the mirror passes into the condenser *at one edge only*, and so impinges very obliquely *upon the specimen* as shown in Fig. 118. If such a diaphragm be so placed (in the foreign models of microscopes this is readily effected by shifting the partially or nearly closed iris diaphragm *away* from the centre of the field to its periphery) and the eyepiece is removed, the observer will perceive, on looking down the tube at the back lens of the combination, a small illuminated elliptical or oval-shaped portion of the field to be the only illuminated area, all the rest being in darkness (see Figs. 120 and 121 showing the illuminated areas in different parts of the field of the back lens of the objective).

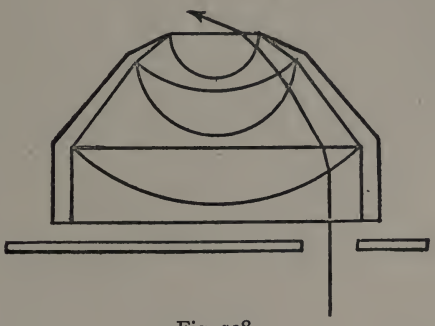


Fig. 118.

Prof. Abbe has explained in his theory of microscopical vision, which is now held so firmly notwithstanding the doubts brought forward in the past by those who by inadvertence did not thoroughly grasp his meaning, that the final image of a small object is formed by the union of two or more differently formed beams—the directly transmitted one from the illuminant on the one hand, and on the other by those varying in number according to the arrangement of the minute structure of the object caused by diffraction (interference phenomena).[1] The union of these differently formed beams brings out the definition of the minute structure of the object. The importance of such blending or union is shown by the fact that if the diffraction effects are artificially stopped out, so that the ocular deals only with the central or dioptric beam, the minute details of the object are *immediately lost*, and the objective seems all of a sudden to have lost its power. It should be borne in mind, we

[1] These are also called the diffraction spectra.

should mention in passing, that with coarse objects, however, such is not so *evidently* the case, or anyhow may not be so apparent; but of this we have hardly time to speak in this place, for the details of the formation of the microscopic image are fully gone into elsewhere in this book. To put, however, our assertion to the proof that these diffraction images *do* enter into the formation of the image with such a potent effect, let the following experiment be performed: Place on the stage a specimen of Pleurosigma angulatum, use a twelfth homogeneous objective, focus and adjust for critical light, centrality of condenser, and so forth. Remove now the eyepiece, having closed the iris to a sensible amount, and look at the back lens of the objective. A central brilliant dioptrically formed white image of the iris will be seen surrounded by six diffraction spectra *coloured red outside and blue nearest the centre of the field*, as in Fig. 119.[1] If now we change the slide, and use in its stead a grating—ruled lines of different distances apart—one being especially sold by Carl Zeiss for this particular experiment, we shall soon discover that the spectra always lie *at right angles* to the objects forming them, and that the *nearer* the lines are ruled together the *further* away from the centre do the diffraction spectra become. Further, if we used different coloured lights—say violet—we should find this distance in question *less* than if we used red; in other words, the shorter the wave-length the less the distance. More as a convenience for future reference than any other purpose these three observations are here set forth.

Fig. 119.

(i) The two diffraction spectra lie at right angles to the objects forming them, *one on each side* of the dioptric beam.

(ii) The nearer the fine structures lie together (such as dots or striæ in diatoms), the further the diffraction spectra are placed from the centre of the back lens where the dioptric beam is seen.

(iii) The shorter the wave-length of the light used, the shorter the distance of the diffracted spectra from the dioptric beam.

In the case of Pleurosigma angulatum, inasmuch as the dots

[1] In Fig. 5, Plate I., a photograph of the back lens of an objective of N.A. 1·40 is shown with the spectra *in situ*.

or hexagons happen to be separated by just the correct distance for the purpose, *both* the direct light and the spectra can be seen in the field of the back lens at one and the same moment (Fig. 5, Plate I.), hence it follows all are blended together to form the image *as seen in the ocular*. For the purpose of again experimentally proving the utility of such fusion, let a diaphragm be made of the correct dimensions and placed over the back lens of the twelfth, so as, in fact, *only to permit the passage of the central or direct beam*; on looking through the ocular the minute structure of the diatom will now be found to have disappeared. A little further consideration is sufficient to render it evident, if the back lens were too *small* in diameter—which is only another way of saying if the N.A. were insufficient[1]—to transmit these *diffraction* spectra, then of course the combination would be unable to show the minute structure of the diatom.

For the purpose of explaining the exact nature and use of oblique light, it will be convenient, before proceeding further, to make an additional experiment. Let the specimen be changed to one of Amphipleura pellucida, and for future convenience let it be placed with its greater length from above downwards in the field of view. It is to be understood we continue using the same objective, but add to the existing details an oiled 1·35 condenser (achromatic). If the N.A. of the objective be 1·40 and the lens a good one, more especially in the correction of its outer zone, it will be possible with a low-power × 6 ocular to see just faintly visible the transverse lines—as we have pointed out later on when considering test-objects for an objective of the focal length in question.

Place now an eighth moon-shaped diaphragm, such as represented in Fig. 120, under the illuminator (or if using a Zeiss or other Continental stand, close partially the iris diaphragm and shift it across the field to produce the same effect), and the transverse lines should now start out into great distinctness (Fig. 2, Plate IV.), *provided* that the specimen be a well-marked one and that the eighth moon-shaped diaphragm *lies at the lower*

[1] Numerical aperture may be said practically to be the ratio of the semi-diameter of the back lens of an objective to its focal length; hence the smaller the diameter of this lens, the less the N.A. of the combination if the focal length remains constant.

portion of the field, as shown in Fig. 120, which represents the appearance presented by the back lens of the objective.[1]

Rotate the diaphragm a quarter of a turn to make it change its position similar to that shown in Fig. 121, and then, returning

Fig. 120.

Fig. 121.

the eyepiece, the *longitudinal* lines as they are called—which run along the diatom *lengthwise*—should be seen.[2] Lastly, change now the position of the diaphragm once again, so that it occupies a position midway *between* those shown in Figs. 120 and 121; and if pure green or, better still, pure blue light be employed, the dots should be visible, provided the specimen be a good one for the purpose. See Fig. 5, Plate XII.; Fig. 1, Plate VI.; Figs. 5 and 6, Plate XIII.; and Fig. 2, Plate XV.

Presuming the reader has followed these remarks by trying the actual experiments, he is in a position now to understand the explanation that follows. If, whilst the transverse lines of the amphipleura are faintly seen when using the lower-power eyepiece and employing a solid cone (without any oblique light), the ocular be removed and the substage diaphragm be closed the veriest trifle, the appearance seen in Fig. 122 will with some difficulty be recognised.[3]

If one of the diffraction spectra (here, as above stated, we refer only to the blue portion) on either side of the central could have been entirely got into the field of the back lens of the objective,

[1] Of course this means that the crescent moon opening is at the top or opposite side *of the condenser*, its lower portion being covered up.

[2] It should be mentioned, however, because of their closeness, it is often difficult to see them when white light is employed, but they should be clearly visible when green illumination is used. See Fig. 4, Plate XII.

[3] The edge of the lower diffraction spectrum is extremely difficult to see, and the eye must be shifted about before it will see the upper one.

then the lines of the specimen would have been well defined; but inasmuch as it is absolutely impossible to bring more than a small portion of it into view, this accounts for the faintness and imperfection of definition to which reference has been made.

Fig. 122.

Fig. 123.

Now what happens when oblique light is used? Practically it is simply this, it means a shift of the central dioptrically formed beam, in company with the diffraction spectra, *further across* the field, as shown in Fig. 123, which permits in consequence, *when the iris is more closed, both central beam and diffraction spectra* to be taken up by the eyepiece completely and entirely at one and the same moment. By doing this the definition is rendered as perfect as the construction of the zones of the objective will admit, constituting another proof of the correctness of the Abbe theory of the formation of the microscopic image.

The same explanation applies when the observer looks at the longitudinal lines; but because such lie (it is usually found) somewhat nearer together in most specimens, so by proposition (ii) their spectra should lie nearer the edge of the field when the dioptric beam is centrally placed in the back lens, Fig. 124. Putting this in other language, as they lie *further from the dioptric* beam than those which come from the transverse markings, so they

Fig. 124.

are more difficult to see unless the microscopist uses a little more oblique light—that is to say, employs *a little further "shifting"* across the field—to get them within its limitations (Fig. 124).[1]

Now as to what happens when the dots become visible. If the reader has experimented he will have found it is not easy to locate the exact position for the direct beam to occupy in the area of the back lens, so that the diffraction spectra from both *longitudinal and transverse lines* shall be present at one and the same moment in the field.[2] To facilitate this operation it is mostly necessary to employ blue light or certainly a blue-green, because by employing the shorter wave-length it lessens the distance of the diffraction spectra from the central beam by proposition (iii), and hence gives a better opportunity of squeezing, so to speak, all three beams into the limit of the back lens (see Fig. 125).

Fig. 125.

The real use then of employing oblique light to show the dots is simply this: to enable the microscopist to get the two spectra required, and also the central or direct beam, into the back lens of the objective *at the same moment*, so as to be utilised by the ocular for the final building up of the image as seen by the eye.[3] From what has been said, the necessity of having a large aperture to the back lens, in other words of having a great aperture to the lens in use, is very apparent, an addition of even ·05 N.A. being possibly of a little service in perfecting the image. It further shows, with low-power objectives (seeing the addition of the diffraction spectra with them is not of such

[1] The student should compare Fig. 124 with the preceding. Whilst recognising both are diagrammatic, the dioptric beam is readily seen to have been sensibly shifted a *further* distance from centrality in one figure than in the other, although the diffraction-beams are not shown to have moved quite as much as they should be.

[2] If the microscopist be using an instrument that is provided with a circular stage that can be turned around the optical axis, it is easier to move the specimen than interfere with the substage arrangements, as by this means the illumination of the field is not interfered with.

[3] In Fig. 6, Plate I., a photograph of the appearance presented by the back lens of a N.A. 1·40 combination is shown when the "venue" is changed, a Pleurosigma angulatum being used.

paramount importance, owing to the details of a specimen used with such a lens being much larger and further apart), the advantage of using oblique light is not so readily felt.

The explanation of this difficult subject—the formation of images and their improvement in detail effected by the use of oblique light—has been written in a manner, it is hoped, that will suit the taste of and be understood by the lay reader; but to those who prefer an explanation expressed in perhaps a more philosophical way, and certainly in more technical language, the following rendering may more directly appeal.

The law governing diffraction by regular periodic structures is such that, after the direct and diffracted pencils have passed through an aplanatic objective, the distances and angles between the several pencils remain constant, no matter how the direction of the dioptrically formed beam is changed. So true is this that the distribution of light seen on looking down the tube could be reproduced exactly if we were to cut out the complete diffraction spectrum of the structure in a piece of cardboard (provided the proper scale to suit the focal length of the objective were maintained) and shift it about over the back lens of the objective. Any combination of openings which can be got that way corresponds to a combination of diffraction spectra obtainable by using appropriate oblique light. To understand this more fully, Fig. 126 may be consulted.

Here we have represented the image seen in the back lens of an objective with unlimited numerical aperture. The numerous spectra (although even all are not shown which theoretically exist) are set out in diagrammatical arrangement, as if the Amphipleura pellucida were under examination; but it must be distinctly understood, of course, that such an ideal light-grasp is impossible. The circle A may be taken to represent (rudely) the area of the back lens of an objective with a N.A. 1·40, X showing the central beam. The position of the circle shows that, with the central beam *centrally placed* in the field, a portion of each diffraction spectrum of the transverse lines is all that is contained within the prescribed limits. This accounts, as we have before indicated, for the lines being very imperfectly or faintly shown when using the low-power eyepiece. They may not have been seen at all if the outer zone of the objective were too small or not well

corrected. This explains, too, with a poor specimen of the optician's art, or one with a low numerical aperture, why it is the observer has such difficulty in persuading himself there are any markings at all, the diatom appearing without structure.

Position B shows the "venue" changed and oblique light in use. One diffraction beam is now noticed to be entering in the field at the same time as the central, so the transverse lines should be well shown when the eyepiece is in use.

Position C explains the situation when the arrangement is changed and the "venue" made for the purpose of showing the longitudinal lines, and it further explains why it is always more

THE THEORY OF OBLIQUE LIGHT DIAGRAMMATICALLY REPRESENTED.

Amphipleura pellucida on the stage and a 1·40 N.A. objective in use.

X represents the central or dioptrically formed beam, whilst the black circles are supposed to be the diffraction spectra, the blue end being turned in all cases *towards* the central beam X (see Fig. 119).

Circle A.—The appearance presented at the back lens of the objective when green light is used.

Circle B.—Oblique light when used to show the *transverse* lines.

CircleC.—Oblique light when arranged to show the *longitudinal* lines.

Circle D.—Oblique light when set to show the dots: two spectra (the blue ends) being in the field at one and the same time.

Fig. 126.

difficult to see them also; because the spectral beams, it will be noticed, being considerably further away from the central one,[1] entails the use of so much more oblique light (or so much more shifting of the "venue").

Position D indicates the position chosen for displaying both lines, which means displaying "the dots." For this purpose both spectral beams, or as much as possible of each, with the central beam must be in the field at one and the same moment of the back lens. As seen in the diagram, it will be noticed this has not quite been accomplished, because the light supposed to be in use at the time has too long a wave-length; hence

[1] On account of the lines being closer than the transverse.

blue or blue-violet must be employed to shorten the distance between dioptric and diffraction beams (iii), thereby allowing their more complete passage through the limited area of the back lens of the 1·40 objective. When this takes place, but not until, can the "dots" be seen. The study of the circle shown at D is very important, and one that must be borne in mind by the microscopist.

This concludes the explanation of the theory involved by the use of oblique light. In practice some little care must be employed, lest by too rapidly changing the position of the semi-lunar diaphragm the exact position may be lost for the complete fulfilment of the necessary conditions. It is an open question whether it is better to have the half-moon diaphragm a large arc of a small circle, or a small arc of a large one. In the latter case a portion of the outer part of the intermediate zone of the back lens of the objective is usually included with the outer; whereas in the former the outer zone is for the most part alone employed. It is evident from this that the advantage between these two cases depends to a large extent upon the relative merits of the outer and middle zones. As a practical guide to the student, what has seemed to us the best method of dealing with the subject is to put both conditions to the actual test of direct experiment. It is not a little curious also that objects of different types often appear to require slightly different treatment; but, whatever the amount of oblique light demanded (which, of course, largely depends upon the closeness of the details), it should not be allowed to produce "effects" in the field of view actually outside the object, lying in space! Further, it is our opinion, seeing that the introduction of false (diffraction) phenomena is very easily introduced into the final image, no details should be admitted as *real* unless they can be seen, although perhaps imperfectly, with direct light, or at any rate with but a small amount of oblique illumination.

The reader may now very naturally ask if it be possible to obtain greater resolution by any means than that of oblique light used as we have described. To answer this question, we must remind him that increase of resolution can only be obtained in two ways—either by making the N.A. greater, or by making the wave-length smaller; hence the improvement so much desired can only be effected by employing one

or both of these two expedients. Up to the present the first is that which has been mostly adopted ; but it is well known by the veriest tyro that a limit has been reached for practical purposes by lenses of N.A. 1·40. Here we do not forget the N.A. 1·6 objectives made by Zeiss with this laudable object in view ; but as these lenses require the whole system, *immersion fluid, condenser, slip, cover-glass, and mounting medium,* to be at least of the same high index of refraction, a restriction is placed upon the active employment of such a combination for all practical purposes, if for no other reason, upon the ground of expense, for we have been informed the cover-glasses—usually made of flint—cannot be prepared in the ordinary commercial manner, but have to be ground and polished by hand. Consequently the only improvement possible was to use light of shorter wave-length than green. It was for this reason much time was spent in perfecting a prismatic arrangement described on page 170. It turned out of considerable service. The outcome was a suggestion that if this slight extra shortening of wave-lengths yielded improved results, why not use light of shorter wave-length still—namely, the ultra violet rays? Many difficulties arose in making the experiment. First, as no glass transmits these undulations, all the optical parts had to be made in quartz which does do so. (These lenses were constructed by Zeiss, and were commercial in pre-war times, see page 61.) Then the next difficulty which had to be met was the fact that the human eye cannot see images in light of shorter wave-length than blue or blue-violet, so arrangements had to be made to photograph them instead. The focussing difficulty was met by employing a screen, much after the method used with X-ray work, which, by lengthening the wave-lengths for the time being, enabled objects to be seen sufficiently well to focus them on the ground-glass of the camera. Unfortunately, the photographs produced by this costly arrangement did not appear to present any great advance upon those obtained by ordinary means when blue illumination was employed.

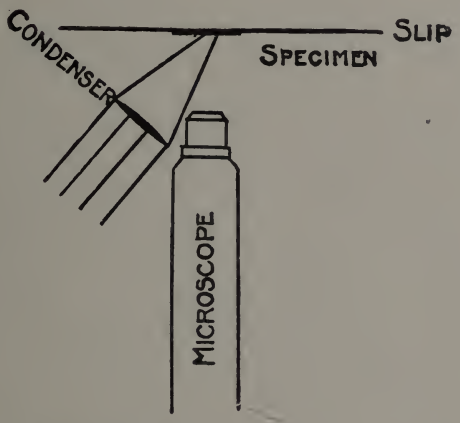
Annular Light

Although we do not recommend this form of illumination, some interesting remarks will be found in the Addenda.

The Illumination of Opaque Objects

Most objects prepared for the microscope are examined by transmitted light; this, however, is not possible in certain instances, on which account their illumination must be effected by some other means. Of these there are several.

The first is by the simple method of casting the light of the lamp or other illuminant upon them, as shown in Fig. 127, which in plan indicates the portion of the lamp and specimen



ARRANGEMENT IN PLAN OF ONE METHOD OF ILLUMINATING OPAQUE OBJECTS

Fig. 127.

with a bull's-eye[1] condenser interposed. There is some difference of opinion which side of the plano-convex condenser should be turned towards the specimen, but theoretically, the plano side should always be turned towards the shorter conjugate to obtain the best results. We have frequently tried both positions of the condenser, and have found that in actual practice it is far better (although this statement may shock some readers) to try by direct experiment which gives the better result. It should be mentioned, however, what dissimilar effects may often be

[1] A reference to the different kinds of bull's-eye is given in a previous chapter.

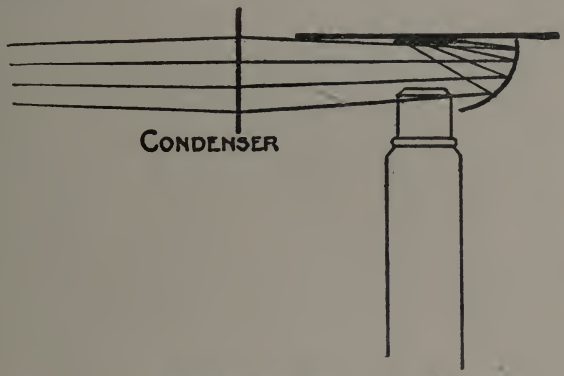
produced by using different angles for the situation of the illuminant, a point which we have never seen noticed before. It is well known that the mountains of the moon show up much better at the first and third quarters, when the light from the sun falls obliquely upon them, than when they are illuminated by beams falling almost, if not exactly, at right angles upon the surface of the moon. A little shadow effect then seems to help to delineate and bring out the mountains very considerably. So it is with certain low-power objects under the microscope. When the light is placed so that its beams fall upon the object in a direction almost parallel with the stage, the effects appear to be very different from when it falls upon it almost vertically. Hence we make it a rule always to try to see whereabouts is the position that gives the best effects by the simple means of direct experiment.[1] The position, however, in this particular type of illumination is necessarily very often limited to a great degree by the mounting of the low-power objective in use at the moment, for some of the older make are so blunt-ended, and of so great a size, that the light can get to the object only when the lamp is in one position. This can be avoided by using objectives of the more modern manufacture that are not so constructed, having their brass mount tapering off to the front lens. On this account we are fond of employing, for the purpose in question, the Holos objectives sold by Messrs. Watson & Sons, or combinations made after the same fashion, as their formation is such that the front lens is itself carried downwards away from the brasswork in a conical fashion, and to such a sensible distance as to permit the light being thrown on the specimen with much greater ease than with many an objective otherwise mounted.

Sometimes the illuminant is so hot that the rays focussed by the bull's-eye upon the object will actually burn or injure the specimen when the examination is a protracted one. It is best then under these circumstances to interpose a water-bath, an exceedingly handy, cheap, and small arrangement being that designed by Mr. Kingsford and shown at the Quekett Club some years ago. It consists of two plates of glass separated by rubber stops and surrounded to a greater part of their

[1] This will determine very often which type of illumination is the best to select out of the four mentioned in this chapter.

circumference by a rubber ring, supported by a strip of brass capable of being tightened by screws. Easy to fill, easy to empty, and easy to clean are very great recommendations. They can be fitted to the ordinary bull's-eye stand, or supplied on stands of their own. These are more fully explained in the chapter devoted to "Accessory Apparatus." Not too large a one serves the purpose in hand, for, if the water gets too hot, it can be easily emptied out and fresh put in, remembering that it is always best to use water that has been boiled and subsequently cooled, because of its containing so much less air. This bath had better be placed between the illuminant and the condenser, rather than between the condenser and the specimen, as there is usually too little room to spare in the last-mentioned situation.

The above-mentioned arrangement usually answers very well with low powers; but when using a magnification of between 200 and 300 diameters, there is great difficulty in illuminating the object at all. A plan suggested by Mr. James to get over the difficulty is worthy of a trial. It is that of placing the light *low down*, and the bull's-eye condenser *so obliquely* that it casts a wedge-shaped beam *along the surface* (or very nearly) *of the*



ARRANGEMENT IN PLAN OF A SECOND METHOD OF ILLUMINATING OPAQUE OBJECTS

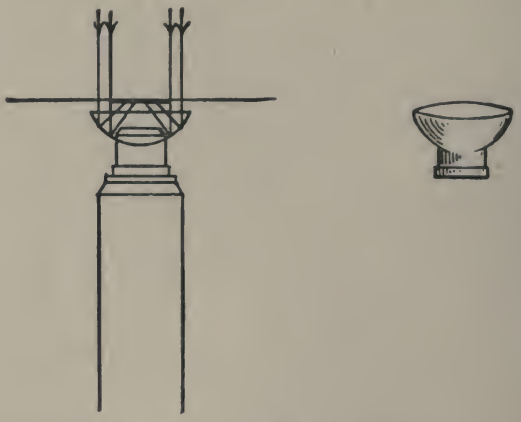
Fig. 128.

stage, just passing between the front lens of the objective and the specimen.

A second method is a modification of what we have called

the first. The lamp is placed so that its rays, after leaving the condenser, pass in a more or less parallel beam[1] along the surface of the stage. On the opposite side of the specimen these are received by a silver parabolic reflector which, placed in the correct position found by experiment, returns them on to the object (Fig. 128).[2]

Another modification constitutes a third method of illuminating an opaque object. This time it is to have a parabolic mirror, with a hole in its centre, through which the front lens of the objective just protrudes (see Fig. 129). The light passing



ARRANGEMENT IN PLAN OF A THIRD METHOD OF ILLUMINATING OPAQUE OBJECTS BY MEANS OF THE LIEBERKÜHN

Fig. 129.

upwards *through* the slip *around* the specimen—which implies that the object must be mounted as if for use with transmitted light—falls directly upon this mirror, which returns it to focus

[1] How to obtain parallel beams from a lens is fully described in earlier parts of this book, page 11.

[2] Since the publication of the first edition of this book, Mr. W. B. Stokes, late Secretary to the Quekett Microscopical Club, has suggested a form of side reflector which is worthy of mention. It consists of a cheap magnifying glass cut in half, with one side silvered. When suitably placed, and held in the proper position, it is said to be quite as effective as the more expensive parabolic side reflector above mentioned.

on the object, an adjustment being provided to assist it in so doing.

The above arrangement furnishes most excellent results, but unfortunately can only be used (if the best possible effects are sought after) for the special objective for which it is constructed, as a longer or shorter focal-length lens will need a mirror of longer or shorter focal length to match, without which the object will not be properly illuminated. The mirror is called a Lieberkühn, after its inventor.

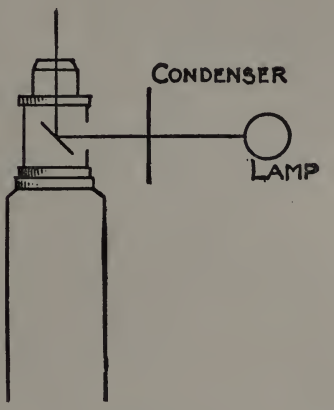


Fig. 130.

A fourth method of illumination, effected in two different ways, is by what is called "vertical illumination." The principle involved is the same with both arrangements, and consists in utilising the objective as its own illuminator. A beam of light enters either device through a little hole guarded by a diaphragm at its side, the perforated brass mount serving to hold the objective at one end whilst it is attached to the nosepiece by the other (Fig. 130). In the case of one arrangement the light then falls upon a

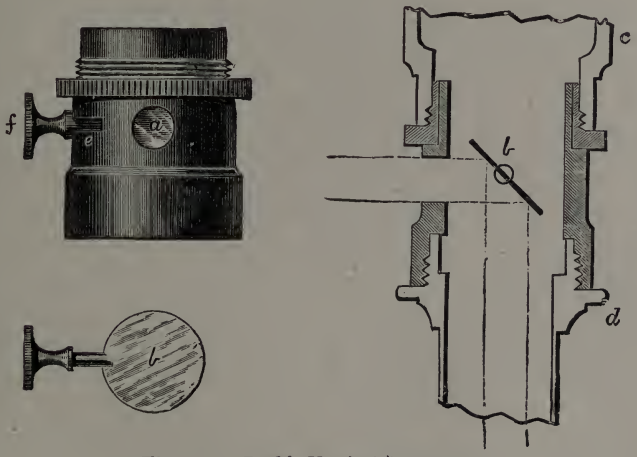
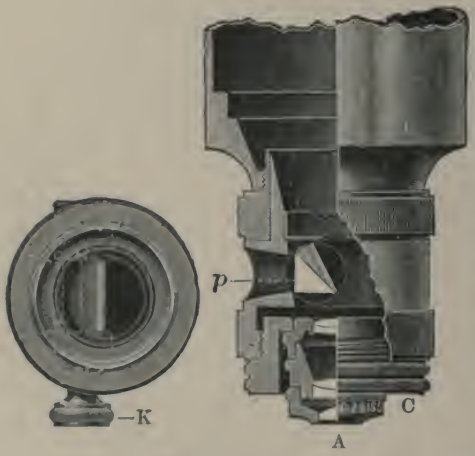


Fig. 131.—Beck's Vertical Illuminator.

Fig. 132.—A Vertical Illuminator by Watson & Sons.

cover-glass placed at an angle of 45° (Figs. 131 and 132) to the optical axis of the microscope (suitable adjustments being provided), by which it is reflected into the objective and from thence on to the object; whilst in the other (Fig. 133) it falls on the hypotenuse surface of a prism designed to cover only half of the objective, passing from there by total reflection into the objective, and from thence, as before, on to the specimen. The first variety offers but little difficulty in use, but the latter needs special objectives arranged with very short mounts, whilst with both types it is needful to employ specimens devoid of cover-glasses. Unfortunately with dry powers



A, view (partly sectional) attached to the lower tube and the objective C, the latter in short mount; *p*, reflecting prism; B, plan view; K, milled knob for revolving the prism.

Fig. 133.—Zeiss Vertical Illuminator (Full Size).

this implies their special correction for the purpose, which means expense; but with oil immersions a cover-glass can be used with both forms of arrangement.

The use of either of these illuminators requires particular care in adjusting the details. It is very obvious that the light has to be directed into the little aperture in the side of the mount with the greatest exactitude. To perfect the illumina-

tion and to furnish enough light, the rays from the illuminant are gathered by a bull's-eye condenser and cast directly through the aperture on to the reflector. Leitz attaches a condensing lens to his latest pattern, which is a convenience.

The great objection to all these arrangements is that, after the light is adjusted, no movement of the tube for focussing is permissible, as it upsets the evenness of the illumination. Messrs. Watson & Sons have, however, introduced a new form, illustrated in Fig. 134, where it is shown in detail. An incandescent lamp is fixed into the apparatus (already provided with a little condenser)

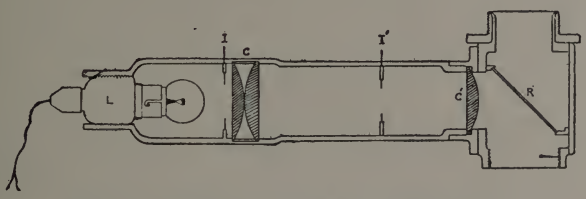


Fig. 134.—Watson-Conrady Vertical Illuminator.

which illuminates the field. It is evident no movement of the tube upsets the working of this arrangement. A little battery provides the current, but a lamp can be supplied for use with the ordinary house supply if specially required.

In metallurgical microscopes described hereafter, the difficulty is met by making the stage move up and down, hence *any* adjustments of focus can be made with impunity.

Polarised Light

One of the first questions asked by the amateur when he sees the beautiful rendering of objects by this peculiar method of illumination is, What is meant by the term Polarised Light? The answer to this is not at all easy, and it is very remarkable how most text-books on the subject of the microscope shirk any explanation. This very probably arises from the fact that to reply to it briefly is so difficult, and to do so thoroughly involves too much space. Perhaps no more concise description and explanation of what is meant by the term exist than that given by the late Mr. Spottiswoode in his fascinating volume devoted entirely to the subject in the Nature Series, from

which we quote the following in the celebrated author's own words :—

> It is considered as established that light is due to the vibration of an elastic medium, which, in the absence of any better name, is called the ether. The ether is understood to pervade all space and all matter, although its motions are affected in different ways by the molecules of the various media which it permeates. The vibrations producing the sensation of light take place in planes perpendicular to the direction of the ray. The paths or orbits of the various vibrating ethereal molecules may be of any form consistent with the mechanical constitution of the ether ; but on the suppositions usually made—and none simpler have been suggested—the only forms possible are the straight line, the circle, and the ellipse. But in ordinary light the orbits at different points of the ray are not all similarly situated ; and although there is reason to believe that in general the orbits of a considerable number of consecutive molecules may be similarly situated, yet in a finite portion of the ray there are a sufficient number of variations of situation to prevent any preponderance of average direction. This being assumed, the process of polarisation is understood to be the bringing of all orbits throughout the entire ray into similar positions.

Even this elegant description, however, may not thoroughly explain the matter to the lay mind, for it requires, anyhow, a certain, though very elementary, knowledge of the subject of light in general, not perhaps possessed by a reader whose attention has been previously directed entirely to other matters. To him, then, the following remarks, though perhaps not so scientifically accurate, may more directly appeal.

With certain exceptions—uni-axial and bi-axial crystals, for example—it may generally be stated that ordinary white light consists of vibrations of the ether in all azimuths—that is to say, in paths in all directions, *each and every one of which are directly at right angles to the path of the ray itself.* This is shown in Fig. 135A, which rudely represents the section of a ray of light coming to the eye. Plane polarisation consists in the arrangement of all these azimuths, *or paths of vibrations,* into *one plane only*, as in B or C in the same figure. An object viewed by light of this description would only appear as if illuminated rather more feebly than usual ; but if examined in a particular manner to be hereafter explained, and with the special apparatus about to be described, certain beautiful and curious results, phenomena most gorgeous to behold, would be exhibited, and a specimen so illuminated and examined is said

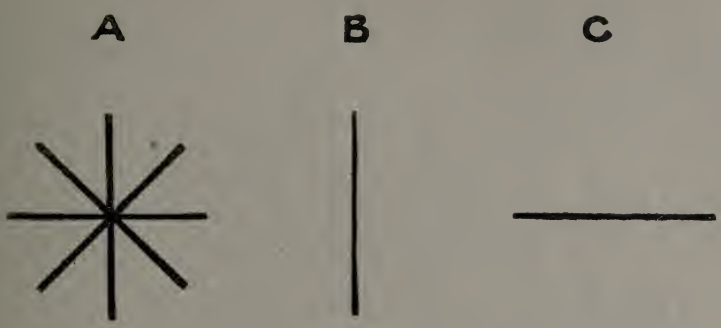


Fig. 135.

to be viewed by *plane polarised light.* If, however, a still further alteration is made in the apparatus employed, as fully explained later on, the object may be viewed by polarised light of a different character, because in this instance the vibrations, instead of being reduced to oscillate in *one plane*, are made to move in a *circular orbit.* An object seen in this manner is said to be viewed by circular polarised light.

It is not desirable to complicate the description of the subject any further, but we should not omit to mention that certain crystals possess the peculiar property of reducing all the azimuths of common light into *two* planes, each at right angles to the other, as, for example, a crystal of Iceland spar. As will be seen in the footnote furnishing a description of what is called a Nicol's prism,[1] the aim of this arrangement is to eliminate

[1] A Nicol's prism (Fig. 136) is constructed from a rhombohedron of Iceland spar thrice as long as its diameter; one of its faces, which

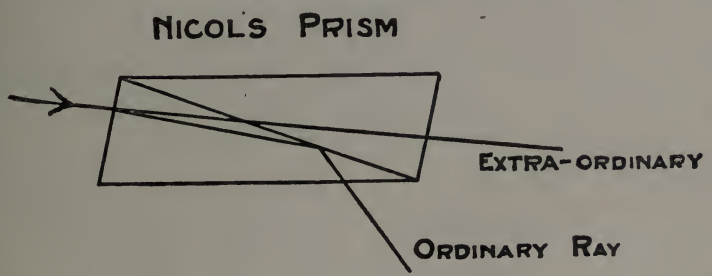


Fig. 136.

naturally makes an angle of 70° with the blunt edges, is cut off obliquely so as to give the new face an inclination of 68° instead. The whole block

one of these, and so to permit the passage of rays polarised in *one* plane only.

The subject scientifically treated, though extremely interesting, is too deeply complicated to be thoroughly explained in a book of this description, and indeed requires much mathematical and philosophical reasoning; but the *use* of these special forms of illumination, and how they are best arranged and produced for the purpose of the microscopist, forms the subject-matter of what follows.

To produce the first effect—viz. the formation of light polarised in *one* plane—a Nicol's prism is mostly employed, for it is more convenient than any other method.[1] This prism, called the *polariser*, is placed and held in position beneath the condenser between it and the mirror by various means, according to the ingenuity of the optician. In some forms the arrangement is constructed to revolve within its mount or fitting an entire revolution about the optical axis of the instrument, such being effected by the use of a milled collar band placed outside; whilst in other cases it is fixed. In any case, the apparatus is so made as to drop into a sleeve or such-like device so loosely that it permits of a slight amount of rotation sufficient for the purpose of adjustment. The polariser should not be unduly

is then divided in a special manner with relation to the axis, the two portions being afterwards united by Canada balsam. A ray entering the new prism is divided by it into two, each in a different plane of polarisation, the *ordinary* and the *extraordinary*. But the refractive index of Canada balsam is 1·54, intermediate between that of the ordinary (1·65) and the extraordinary (1·48) rays respectively. Hence the most refracted ray finds in the layer of balsam a *less* refracting medium, and is *totally reflected to one side*, and so for most purposes practically got rid of; whilst the other finds in the balsam a *denser* medium, and therefore passes through. This is the ray used.

[1] Light can also be plane polarised by reflection off polished surfaces provided the incident beam makes the correct angle with the perpendicular to the reflecting surface. This is called the "polarising angle," and varies with different substances: glass being 54° 35′, water 52° 45′, quartz 57° 32′, diamond 68°, and for obsidian—a kind of volcanic glass often employed for this purpose—56° 30′. A bundle of thin glass plates is sometimes used as a polariser instead of a Nicol prism, but is inconvenient; also a piece of polished opal glass as suggested by Mr. Michael; whilst a circle of black glass has lately been recommended by Prof. Cheshire. This is set at the correct angle, and slides on to the tail-piece instead of the mirror.

small in diameter, for if it be so it will cut off too much of the light issuing through the condenser; hence if high powers be employed the consequent loss of aperture and illumination will be distinctly felt. Still, however, it should not be too large, for it will then be cumbersome and inconvenient, and should rotation be employed the fingers will keep knocking against the mirror, causing thereby considerable annoyance. The light issuing from the prism (the polariser) is, we have already said, what is called "plane" polarised, but for the observer to be able to witness the peculiar phenomena spoken of, it must traverse yet another nicol before reaching the eye. This is called the *analyser.* Although the principle involved in the action of this second prism is similar to that of the polariser, still its actual shape is somewhat different, according to which position it occupies, viz.—

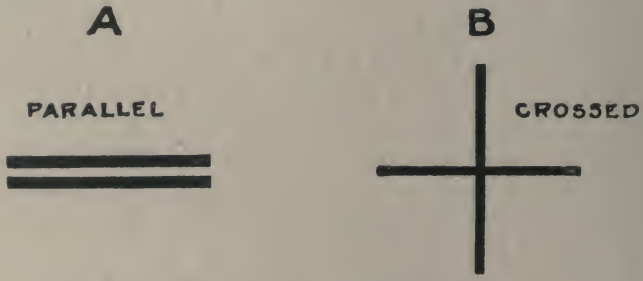
(i) In the microscope tube directly above the objective, (ii) as a rotating cap over the ocular, (iii) or as a combination with lenses to form what is called Abbe's analysing eyepiece, which is used instead of an ocular.

(i) When arranged to fit into a cell to work above the *objective* it is short, and the mount is attached to the nosepiece at one end and receives the objective at the other, a milled collar serving to rotate the prism around the optical axis.

(ii) When constructed to be used over the ocular as a cap (Prazmowski prism), the spar is shorter still and cut somewhat differently; rotation being effected by revolving the whole cap and prism about the top of the ocular. With this kind and with the previous one, almost any ocular of any power, whether Huyghenian or compensating, can be used. A tourmaline may be used for this purpose and in the same manner, but its colour is sometimes objectionable.

(iii) Abbe's analysing eyepiece, which is difficult to obtain now even second-hand, consisted in pre-war times of a specially constructed Nicol with flat ends, placed between the lenses of either a No. 2 Huyghenian or a X4 compensating ocular. But we have found that if the field and eye-lens of the No. 2 Huyghenian analysing ocular be removed, the X8 or the X12 compensating ocular can be screwed into the upper end of the analysing eyepiece, no field lens being required at all. It is a delightful arrangement to use.

It will be noticed from what has been said, that either polariser or analyser can be made to revolve with equal effect; in fact, it is quite optional which movement is chosen. Should this be the polariser, the fingers in turning it are rather apt to knock against the mirror and so put it out of position; if the analyser, eye-cap form, or the Abbe eyepiece arrangement, the eyelashes may get in the way of the fingers or thumb whilst rotating, whereas if the form that is placed above the objective be employed, *all* these difficulties are removed. There are objections, however, to this type which do not arise in the use of the other two, for the object may appear to *shake* as the prism is rotated, unless the fitting be very nicely made so that it revolves in its jacket



DIAGRAMMATIC REPRESENTATION OF THE PLANES "PARALLEL" AND "CROSSED"

Fig. 137.

very smoothly and evenly; or to travel about the field, if the inside fitting be at all eccentric to the optical axis or the prism badly made. Occasionally the "travelling about the field" is caused by the objective being not quite "true" in its mount.

Using a low-power condenser, and after having placed the mirror in position, and attached an inch objective to the nose-piece of the microscope; on moving the polariser or the analyser around the optical axis it will be immediately found that alternate intervals of light and darkness will be produced. This is caused by the single "planes" of polarisation being parallel or crossed one with the other (see Figs. 137A and B). If now a "polariscopic object"[1] be placed on the stage, given portions of it *may* present beautiful variegated colours (if such portions be of the

[1] This is the name given to objects, or to crystals or such like, that are "suitable" to exhibit *good effects* in polarised light. For a short list, see p. 510.

correct thickness for the purpose), whilst the whole object will always show extremely elegant and attractive, not to say at times very pronounced and beautiful, changes of *black and white effects*, varying from almost blackness to a most pearly, silvery white. All manner of details are brought into evidence by this means and certain conditions are revealed which in many instances are not to be seen by any other method of illumination. For example, badly annealed glass may be detected, and also glass lenses under strain. Certain effects, too, in the growing of seeds may be witnessed by this simple means that are not visible by other methods.

Should, however, a thin slice of mineral called Selenite [1] be placed on the stage *between the specimen and the polariser*, colour changes take the place of the black and white effects just described. As this plate is revolved about its axis, there will be found to be two positions in an entire revolution where a maximum effect is produced. These should be noted by some sort of mark corresponding to one placed on the polariser, so that by putting mark to mark the position of maximum effect can in the future be at once secured without any direct experiment.[2] What colour effects are actually produced depend upon the thickness of the selenite. Plates are commercially sold of two thicknesses—one yielding blue and yellow colours, the other red and green. In the previous experiment, we said it was possible that certain parts of the specimen showed variations in black and white effects ; when the same object is placed on the stage with the selenite beneath it, these black and white phenomena are now changed into modifications and blendings of blue and yellow, or red and green, according to the thickness of selenite

[1] Selenite is a mineral consisting of hydrated sulphate of lime in oblique prismatic crystals. It requires much practice to cut perfectly even slabs of uniform thickness. We have been able to split circles of about half-a-crown in diameter by using a *very* flat and thin knife and keeping the material under water. We believe the finest specimens are ground and polished ; but the method adopted by the trade is kept a close secret.

[2] Lest any confusion should arise in the reader's mind by saying the selenite is revolved about its axis, we might explain that if held between the thumb and finger, one on the front surface and the other on the back, revolution around these fixed points is the movement about the axis we speak of. It is convenient to have the slab *circular* ; its "best position" then can be marked so that the mark can always lie upon the top of the polariser in the same place.

employed. It is obvious then a set of suitable specimens, called "polariscope objects," reveal different colour phenomena with each selenite, hence by this simple means many an enjoyable hour may be spent by those fond of gorgeous scenic effects.[1]

But there is yet another form of polarised light which, when correctly used, gives results so beautiful and entrancing that it puts the phenomena just described quite into the shade. We refer to the use of what we have already called attention to, and which is termed *circular* polarisation, in contradistinction to *plane* polarisation, which we have lately been explaining. The curious feature of this class of polarised light is that, instead of variations in blue and yellow, or red and green, *the colours of the whole spectrum are utilised.* As the analyser (or the polariser) is revolved, one end of the spectrum seems joined up with the other, hence when one nicol is revolved in one direction, blue merges into green, green turns into yellow, yellow into orange, and then red, whilst the union of red with blue, to complete the circle we mentioned, forms a curious plum colour of a most magnificent hue. Reversing the nicol reverses the order of colours. All these changes are brought about by placing above or below the selenite, but beneath the specimen, what is called a quarter-wave or quarter-undulation plate. This is really nothing but a piece of mica, exceedingly thin and mounted in Canada balsam between two thin pieces of glass.[2] A position of maximum effect has to be found by direct experiment for the quarter-wave length mica to lie upon the selenite, and when ascertained, both mica and selenite should be distinctly marked for future use, so as to avoid further trouble.

The best plum-coloured effect, we think, is always obtained by using the quarter-wave plate with a red-and-green selenite rather than with a blue-and-yellow one; but there are very possibly those who do not agree with us in this opinion. Suit-

[1] On revolving the slide *only*, the polariser and analyser being untouched, the specimen itself changes colour, whilst that of the background remains unaltered.

[2] A quarter-wave plate may be made by splitting with a fine needle a piece of talc into thin slices. One or more of these can usually be found that, when placed between two nicols, one of which is rotated, only shows a change of colour from bluish-black grey to fawn-colour. This is approximately a quarter-wave plate.

able specimens used with circular polarised light thus obtained give the most gorgeous and magnificent aggregation of different colour renderings imaginable as the analyser is turned. No artist can depict, or eye picture, such a mixture of shades and changes of colour as may be witnessed by this simple means.[1]

If the light be not equally distributed over the whole field of view—the condenser having been suited to the objective—the other side of the mirror may be tried. High powers do not seem to yield such brilliantly coloured effects, and altogether are not so suitable as low ones.

We used to think the Nernst electric lamp—believed to be unobtainable nowadays—the best illuminant, lamplight being too yellow; but in the Zig-zag, mentioned on pages 166 and 185, we find an excellent substitute.

A very great amusement is furnished to the microscopist in making his own specimens by the simple method of forming concentrated solutions of different soluble salts, and allowing drops of them to crystallise out on slips or on cover-glasses, finally protecting them by suitable means.

Convergent Polarised Light

So far we have only spoken of the use of plane polarised light with and without the interposition of a slab of selenite between the nicols, concluding with an account of a third form of this peculiar illumination called circular polarisation, where, in addition to the selenite, a quarter-wave plate of mica is added: there yet remains, however, to be described a fourth method of employing this interesting means of investigation, one restricted to the viewing of what is called the "rings" and "brushes," which certain crystals exhibit when viewed with the special device we are about to describe. To understand the object of the special arrangement in its component details, it should be mentioned that hitherto we have been explaining the effect produced by the employment of plane polarised *parallel* beams

[1] To prevent complication, it has not been mentioned before that the actual thickness of different parts of the specimen also produces modification of colour-effects as the nicol is revolved. Turning the slide *only* (background remaining unchanged), revolving both nicols simultaneously, moving specimen and *analyser together*, and *specimen and polariser at the same time*, all produce in most instances different effects: especially gorgeous with acetalinide. They baffle description.

of light, showing what marvellous information they unfold of the inner constitution of bodies whether as a revealer of unequal tension—as in the case of unequally cooled glass, for example—or of the different interference phenomena exhibited when layers of unequal thickness of certain substances are successively viewed. Now, however, we wish our apparatus to be so arranged as to show the curious effect that sections of certain crystals exhibit when plane polarised rays of distinctly *convergent* light are brought to bear upon them. These effects are collectively called the rings and brushes. They vary very much in uni-axial and bi-axial crystals,[1] but in either case are very curious, and their interpretation and thorough comprehension offer a considerable intellectual enjoyment. The great object, then, now of the microscopist is to arrange the microscope to obtain these convergent beams in such a manner that they shall fall on the crystals in question, and be viewed in the proper manner. For this purpose the microscope has practically to be turned into a telescope of such a nature that it will deal with wide-angled beams. The first thing necessary is a wide-angled condenser, and we believe in the usefulness of the achromatic variety, although, be it understood, it is not an absolute necessity. The objective should not be of very high power, for we are not dealing with the very minute; but the important point to recollect is that it should have enough and no more numerical aperture than required, about ·65 to ·70 being sufficient.[2] If the objective be of too low a power, although it be of the stipulated N.A., the back lens may be too large to be entirely viewed

[1] In the uni-axial crystals, such as calcite, quartz, borax, sugar, nitrate of soda, ferro-cyanide of potassium, etc., only *one* ray performs in the "*ordinary*" manner: the other is what is called the "extraordinary," which means it does not follow the usual well-known laws. But in the bi-axial crystal discovered by Sir David Brewster *neither* follows the usually well-known laws; both are therefore "extraordinary," the index of refraction varying with the direction of the ray, the refracted ray being not always in the plane of incidence. Such crystals then exhibit a double set of rings and brushes, which, while complicating the phenomena, add very much to the magnificence of the final effect. Some crystals are particularly fine in the phenomena displayed by them. Of these may be selected nitre, native crystals of carbonate of lead, glauborite, and some of the varieties of felspar called adularia.

[2] Strictly, the combination should have its N.A. equal to or exceeding the *angle of the crystal.*

through the nicol ; hence, generally speaking, the best for most purposes is a $\frac{1}{3}$ or a $\frac{1}{4}$-in. The upper nicol may be above the eyepiece, or in the more usual position above the objective. To complete the conversion of the microscope into the ideal telescope, a 2-in. objective[1] (such as the one used with the apertometer will suffice in most cases) should be screwed on to the nosepiece attached to the *draw*-tube, thus constituting one of the uses for this detail referred to when describing this part of the microscope in the earlier part of this work.

For convenience of those using this arrangement for the purpose in question, a diagram (Fig. 138) explaining details is

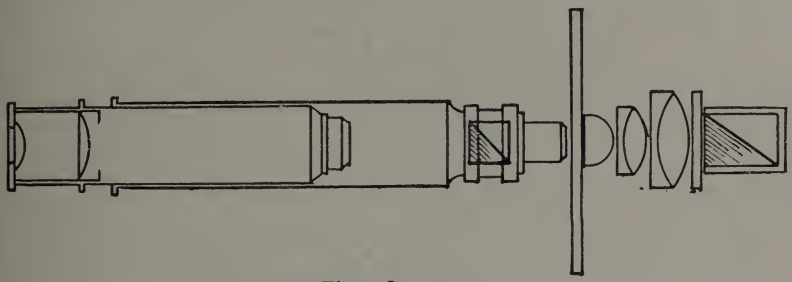


Fig. 138.

given, being followed by the order of procedure to get the best results, which we believe is directly or indirectly due to Mr. Nelson.

1. Fix objective on the nosepiece of the *tube* ; centre condenser and light, and open iris wide.
2. Set the nicols so that a white field is obtained.
3. Add auxiliary 2-in. objective (Bertrand's lens) to the nosepiece of the *draw-tube*, and replace it in the tube in much about its usual position.
4. Place specimen on the stage.
5. Drop the tube (and, of course, with it the draw-tube) by means of the coarse adjustment until the objective (say the $\frac{1}{3}$-in.) all but touches the cover-glass or the crystal.[2]

[1] The form most suitable is a plano-convex or achromatic combination especially made for the purpose and called a "Bertrand's Lens."

[2] *Usually the absence of the cover-glass* produces the best results ; indeed in some instances it is compulsory. In cases where the crystal is mounted dry beneath a cover, the attempt may be made first with it *in situ*, removing the cover subsequently if it be found necessary.

6. Focus *with the draw-tube, leaving the coarse adjustment entirely alone.*

7. It may be necessary to change the position of the substage condenser to obtain plenty of light. It will mostly be found to require raising.

If the analyser or polariser be now revolved on the optical axis in the ordinary manner, the "rings and brushes" will be well seen. Sometimes magnificent and curious effects are produced.

We append the following notes, which may assist the student when commencing to use convergent polarised light:

i. If the microscope in use be one with objective-changers and the analyser placed over the objective in a fitting, and it is found the auxiliary lens will *not* focus the brushes properly,—it arises from such being of too short a focal length. Use one of longer focal length or remove the changers.

ii. If the circle of light as seen through the ocular appears "cut off" in one part which revolves as the analyser is turned, the condenser requires *lowering*, or the nicol polariser may be too small.

iii. If the object be too much magnified, use a lower ocular; a lower power objective is not very successful. If requiring magnification, use a higher ocular.

iv. If the brushes are not shown well, or do not cross the field completely, it is probable the "best position" of the polariser has not been found with respect to the specimen. Turn the former one-eighth of a revolution.

v. A selenite with or without a quarter-wave plate may be used if desired.

With old-fashioned binocular microscopes the above directions are useless, there being no draw-tubes; the following hints will then be found of much service:

i. Convert into monocular, add polariser and inch objective.

ii. Remove eye-piece cap, replacing by same length of brass tubing.

iii. Put specimen on top of this tube, and revolve nicol over it; the "rings and brushes" will be then seen.

It is a convenience to have a fitting made to hold the crystal slide, and which at the same time allows the nicol to be revolved concentrically with the axis of the instrument. A good tourmaline is very useful.

NOTE.—Daylight gives the finest colouring for all polarising effects, but a close approximation is afforded by using a No. 38 Kodak Screen (gelatine) with the electric light. A useful list of substances well suited for polarising effects is given in the Addenda.

CHAPTER X

ON THE USE OF THE MICROSCOPE

EVERY microscopist should endeavour to become master of the instrument in all its uses and in all its details. There are, no doubt, many objectives that have been purchased and thought to be "splendid" at first, which have subsequently been found faulty simply owing to the purchaser not having had adequate experience in the art of testing them at the time of purchase; but there are far more specimens that have been laid aside or rejected as poor and badly marked, because the microscopist has been ignorant of the proper way of centring his condenser or using his objective to obtain the finest possible seeing. As this work is intended to start the actual beginner on his way, as much as indeed it is hoped it may be of service to others more advanced, so the method of using the instrument will be explained in detail even from the very first.

The question often asked by those about to commence the subject, especially those that intend entering the medical profession, is, Which tube-length shall I buy, which is the better, and what is the difference? This is a long matter to discuss, and after all is much a matter of individual opinion. As regards the origin of the two instruments, there is no doubt the long-tube model originated in England, whilst the short-tube instrument had its birth abroad; but its seems doubtful who was the actual originator of either system, although the latter is usually ascribed to Oberhauser.

When first introduced into England the Continental instrument was not at all well received by the majority of microscopists. This arose from the fact that it was then held as an unwritten law that all, or very nearly all, of the magnification of an object should be performed by the objective, and as little as possible by the eyepiece. Hence, as it was obvious to obtain an equal magnification with the objective placed on the short tube as

could and would be obtained if it were attached to the long, it necessitated the use of a higher power ocular; "an instrument, therefore, demanding such a condition of things could not be recommended or tolerated for a moment."

This dictum, however, rested on an entire misapprehension. It is, of course, true that with the *same* tube-length a higher eyepiece puts the objective to a severer test; but the fact was lost sight of that the higher eyepiece, when applied to a short tube, received a smaller, and therefore sharper, image of the object, and that, if the resulting magnification was the same, *the quality of the image was also identically similar*. Hence this objection entirely falls to the ground.

Optically speaking, there should be no difference between the performance, say, of a good $\frac{1}{12}$-in. on either form of model, provided, of course, the actual specimens of the optician's art are of equal merit.

The advantages and the disadvantages of the difference in the size of the oculars in the case of the two instruments is also a question that has often been discussed, without much profit in the end. The increase of field in the long-tube eyepiece (because of its diameter being larger) is of questionable advantage, seeing that such increase is usually more or less fuzzy, the reasons having been explained already when discussing objectives and eyepieces generally. But the principal point of a dissimilarity in the instrument to lay hold of, we think, is the great difference in portability in the one case over the other, and this is especially to the front when instruments have to be carried about, especially by medical men in the tropics, where the distance travelled is often very great. Further, when observations have to be made with the stage *horizontal*, the long-tube instrument often involves the use of a stool rather than a chair, which is of sufficient height with a Continental instrument.

It has been stated also that one point connected with the Continental model has been often overlooked. It is that a smaller difference of tube-length produces a greater effect in the adjustment of objectives than obtains with the longer tube. Although classified as a defect, it is open to consideration as to whether this is not an advantage, as a smaller amount of shift brings about the adjustment required; but it may be argued, on the other hand, that greater care is needed.

Mr. Nelson has additionally pointed out what he considers as yet another disadvantage with a Continental tube-length, and that is, the eye does not see the stage in focus (unless the vision is a short-sighted one) without drawing back the head a sensible distance. As a matter of fact, we personally hold this to be an advantage, for the "off-duty" eye, which should be always kept open, not seeing objects in a good focus, is not so likely to cause a mixing-up effect with what the other eye is seeing as it looks down the tube.

It should not be forgotten, in the comparison between these two stands, that to obtain the highest magnification the English model *must* be employed because of the greater length of the optical tube (and consequently the greater magnifying power produced thereby); whilst as a set-off against this it should be borne in mind that to employ the lowest amplification possible the use of the Continental model is *imperative*, because of the shorter optical tube-length.[1]

[1] This may not be immediately apparent, but it will rapidly be understood by the following argument: All oculars on the short tube apparently magnify 1½ times more when transferred to the long, because the optical tube-length of the objective is 1½ times greater when adjusted for that mechanical tube-length. Hence, when a given magnification is obtained with the most powerful objective and the highest ocular on the Continental instrument, the same ocular and objective (the correction of the latter for the change of tube-length being neglected for the moment) when transferred to the long tube furnish 1½ times the magnification previously obtained on the short one. *Vice versâ*, when the lowest magnification is obtained that is possible on the long tube, this is immediately proportionately reduced by transferring the ocular and objective to the short one. The change in each case, we have said, is not really due to the ocular, but to the alteration of optical tube-length of the two objectives when differently corrected for the Continental and English mechanical tube-lengths. It might be mentioned here that the late Dr. van Heurck, instead of changing the objectives, used what he called his "transformer." This is either a negative or a positive achromatic combination, which added to the back of the objective, either corrects the short to the long tube or *vice versâ*. He used to say that "it corrects in either case so perfectly that it was not necessary to have a double set of apochromatics." We have never seen or tried these transformers, nor have we been able to ascertain any details as to their manufacture; but we should imagine that difficulties would arise in centering these additional lenses, so that the alignment with such delicate and complicated combinations as an eighth or twelfth was not interfered with and the final performance spoiled.

It is evident then, taking all things into consideration, there are advantages and disadvantages with each type of stand, hence we think that the happy mean is struck by employing a stand which by the use of *two* draw-tubes can be employed for both purposes. This can, as a matter of fact, be done with an instrument of Continental length by having a short length of tube made to drop into the draw-tube when required; whilst in the case of the English model any method of shortening it is, of course, impossible. Microscopes are now manufactured with a *double* draw-tube, which enables them to be closed sufficiently for using objectives corrected for the short tube, whilst at the same time they can be extended to suit the correction of those made for the long tube. This then would seem the ideal instrument to select if required for the double purpose mentioned.

Illumination

The ordinary lamp[1] used by microscopists being trimmed and lighted, it is placed with the flame edgeways towards the observer, about six or seven inches from the mirror,[2] the concave surface of which is turned uppermost. The instrument should be inclined at an angle suitable to the observer, so that he can look into the eyepiece without straining his neck. It is a good plan for the actual beginner, in learning how to adjust the mirror, to commence—after removing both the ocular and objective—by looking down the empty tube towards its lower end. There will be seen the mirror. On examination, it will always be found to move very easily in two directions. To learn what these are in the simplest manner, it is best to affix a short length of pencil—say an inch and a half long—by means of a little piece of common candle-wax to its very centre, at right angles to the surface, and with the point directed vertically upwards. The microscope being placed in front of the observer in the usual position, the mounting of the mirror will be found to provide two sets of movement, one of which is termed "the side-

[1] The electric Nernst lamp, which used to be so much employed, is, we believe, not obtainable now.

[2] The distance away of the illuminant is an important matter when dealing with high-power condensers (see p. 238).

to-side" motion, in which the pointer moves in an arc stretching from right to left, or from left to right, as the case may be; whereas the other, called the "to-and-fro" direction, makes it travel exactly at right angles to the former line of motion. · The object of these movements to and fro, and from side to side, is obviously, by their combined use, to find a position that ensures the mirror reflecting the light of the lamp into the instrument in such a manner, after traversing the lenticular portions, it shall reach the eye of the observer. To perform these movements quickly and yet with precision, it is best to place the right arm on the table around the corresponding side of the instrument, the left passing likewise around it on the other side. The first finger and thumb of each hand should grasp the corresponding edges of the metal cell containing the mirror, and then with a little practice the operator will find he can make the light reflect up into the centre of the tube, and so to his eye with great facility.

Most beginners find considerable difficulty in "getting the mirror right," as they call it, and often have to waste much time in so doing; but we venture to suggest, if they will only learn to adjust its position in the simple and primitive fashion above described, *before* attempting to do so with the lens and ocular *in situ*, their subsequent difficulties will rapidly vanish when they get a little more experienced. Having attained proficiency in adjusting the mirror so that the operation can be speedily accomplished, the tyro had better now place an inch on the nosepiece and a low-power ocular in the draw-tube—say a No. 1 of some opticians, or an A eyepiece or a 2-in. of other manufacturers.

In screwing on an objective to the nosepiece, and in taking it off, many a one has been dropped and perhaps seriously injured. It is well then for the beginner at once to learn a method of doing this which will effectually avoid the above-mentioned accident. Let the objective be held as far away from its *screw end* as possible—that is to say, as near as convenient to the end next the small front lens—between the first and second fingers of the right hand, much in the same fashion as a cigar is held between these two fingers, the screw end of the mount representing the portion of the cigar that goes into the mouth. Having previously raised the tube of the microscope by the

coarse adjustment, the screw end of the objective is placed against the nosepiece—screw to screw—being gently held there by the two fingers of the right hand, as already explained, whilst the thumb of the same hand serves to balance and steady it in position. In the interval *between the two fingers and the screw end of the objective*, the thumb and first finger of the *left* hand are now slid in such a manner as to grasp somewhat firmly the milled edge of the mount always present. This must be turned in the *opposite* direction to the movement of the hands of a watch, the microscopist throughout the operation being supposed to be sitting or standing behind the microscope—that is to say, with the instrument in front of him. The right hand should now *gently* press the objective *upwards*, towards the instrument, so as to assist in making the screw of the objective engage into the thread of the nosepiece. If this will not "take in" easily, it is a good plan to draw back in the opposite direction for about half a turn or so, until the thread of the one gives a click as it drops into that of the other. A few turns, and the objective is "home" and in its final position. In removal, the same method may be adopted, but the order of everything is necessarily reversed. Gripping the lens with the left thumb and forefinger around its milled edge, and turning *in* the direction the hands of a watch move, the combination begins to leave the nosepiece. Before allowing it to advance too far, the first two fingers of the right hand should be quickly placed over the free end to hold it cigar-fashion as they did before, which position allows them to prevent the combination falling on to the table or floor should the screw disengage itself from the nosepiece before it was anticipated. This simple expedient to save accidents should be very freely practised over and over again, until, in fact, the operation can be performed almost unconsciously; for, be it understood, dropping an expensive apochromat, for example, often turns out to be a far more costly trouble than at first thought it might appear, on account of the jar being so apt to loosen or displace (if not actually to break) one or more of its numerous little component lenses; an accident that may quite spoil the performance of the combination and necessitate its being sent to the optician to overhaul and repair.

The inch being safely attached to the nosepiece, a specimen—say the proboscis of the blow-fly—should be placed on the

stage in the reverse position to that in which it is desired to be seen,[1] and duly fastened there. If the stage be a mechanical one, the adjustment of the specimen into its proper position beneath the objective is made by turning the screws provided for the purpose; but if it is only a plane one having but a couple of clips, a word or two of advice may be given as to how to shift the slide from side to side, or from above downwards, in the most convenient manner. The *thumbs* of both hands should be employed *upon* the slip, one at one end of it and one at the other, the *fingers* being placed *beneath* the stage so as to get a firm hold. When, on looking into the ocular, the position is found to be correct—the thumbs moving the slide until it is so—the left thumb should press the specimen gently but firmly on to the stage, so as to hold it there, while the right hand adjusts the clip, after which the right thumb does the holding, whilst the left hand completes the fixing by manipulating the second clip. Pressing either of these clips "home" causes them to hold much tighter in case they "work loose." To find the correct position of the specimen, however, or even to find it at all, is often a great trouble to the beginner, or even at times to the advanced student, and sometimes to the experienced worker. The following "dodge" we have often found of great convenience, with dry powers especially. Having placed the inch—for example—as near in focus as can be judged—a little too far off is better than the reverse—the ocular is removed and the head drawn back a few inches, whilst the eye glances down the tube of the instrument. Most frequently the object can be very readily seen, as the thumbs move it about in all directions; it can then be quickly slipped into an approximately central position, to be finally adjusted later on. It is difficult to use this method, however, with high powers. Whilst focussing a specimen, a method equally well to remember is always to lower the objective as near as possible to the cover-glass *before* attempting to look into the instrument, and then, whilst the eye is at the ocular, to

[1] This is often spoken of as "upside down." It is not however a strictly accurate method of speech, for it might be taken as meaning that the "slip" was to be turned next to the objective, and the cover-glass to face the condenser! What is meant is, that if the cover-glass represented a watch dial, the xii should be turned round, so that it occupied the usual position of the vi and the iii that of the ix.

obtain a sharp focus by *raising up* the objective, rather than by lowering it down—the more frequent method. With all microscopists, save those that are frequently at work, this simple expedient saves many a crushed cover-glass or broken front lens. It should be mentioned, however, the method is only of use with dry objectives; with immersion ones another safety plan will be given when their use is under discussion.

Fine focussing of the specimen may now be performed by using the fine-adjustment screw, but before ever attempting to use it the learner should satisfy himself which way the screw turns to lower the objective, and which serves to raise it up. All microscopes are not quite alike in this respect, although in far the majority, by turning the milled head in the direction that the hands of a watch move, the objective is lowered, and on screwing in the opposite direction it is consequently raised. Before leaving this part of the subject, we should like to answer a question that may be very naturally asked by, or anyhow arise in the mind of a reader, and that is why all these precautions should be taken with an inch objective, seeing that it focusses such a long way off the specimen? We reply, in answer to this question, that it is because of the great expediency of getting the beginner into habits of care and caution; for if such be established while commencing to learn the use of the instrument—as indeed was the case when we began ourselves—when the student later on employs higher powers, consequently of shorter focal length, he will follow on in the same lines, having from the commencement learnt that with delicate tools a delicate handling is necessary.

There is yet another piece of advice which may be mentioned here, in want of a more suitable place. It is always to recollect to raise the tube, and consequently the objective, before ever attempting to remove a specimen off the stage, whether it be to replace it by another or simply to put it away. If the latter, it is to prevent the "ringing" of varnish so often present catching against and very possibly scratching the delicate front lens of the combination;[1] whereas if for the former purpose it is, in addition, to save jamming the next slide between the objective and the stage—an accident possibly occurring when the second slip happens to be a thicker one than the first. If now the

[1] Especially the case with high powers, as their working distance is so much less.

tube has been previously raised in either of these cases, a nasty accident is thereby avoided.

To resume, the delicate focussing can be done by the fine adjustment which may be necessary when a high ocular is in use even with an inch, although of not so much service with a low-power one. It is quite likely now that, even with the A ocular, directly the student looks at the proboscis he will recognise that the mirror does not reflect the light equably over the whole field, and hence that it requires a little further adjustment. This should be done with the arms resting on the table as before explained. But perhaps with all this the image is poor; this may arise from the concave mirror requiring a little raising up or down. If the high-power ocular now replace the low one, it will soon be found, especially as the student's eye learns to see and appreciate fine definition, that the very minute hairs of the proboscis do not look quite sharp and clear. They may appear, no matter what the focus, fluffy and with double tips. This is very likely because a substage condenser is required. There are several very good ones in the market, but few seem to know that the "loups" used as hand-magnifiers made by Zeiss and others can be employed as excellent condensers for an inch objective. We repeat that very good low-power condensers are made by most manufacturers, but we must admit to having a great liking for the very lowest one made by Messrs. Watson & Sons after the formula of Mr. Conrady. Its lenses are about $1\frac{1}{4}$ in. in diameter, and its focal length is 2 in., and when mounted it just drops into the substage. It has the advantage, too, that, when not in use in this position, it makes the best bull's-eye with which we are acquainted.

Having placed the condenser into the substage, or in the sleeve which takes its place, between the specimen and the iris diaphragm or its representative, the whole is racked up or down, or pushed by the fingers, until a very perfect illumination is effected. On focussing, the finest hairs should now appear in excellent definition, looking as if they grew upon the stage and were rising up to the eye! Should the light be found too powerful, the condenser may be lowered (permissible with this long-focal length objective) just *a little*, or the iris may be closed a trifle; but it should not be forgotten that both operations, especially with a high-power objective, are apt to spoil definition

and reduce the resolving power of the combination. If, however, the light be still too bright, a thin opal glass may be interposed between the mirror and the lamp, or, better still, one of the many neutral tint filters sold by Kodak: No. 96 is very useful.

When using a $\frac{2}{3}$-in., which has usually about the same N.A. as that of the inch, no difference need be made in any of the operations, only it should be recollected that it focusses much nearer the specimen. Whilst stating this, it is well to recollect, however, that owing to the variety of computations adopted by different opticians, the exact position occupied by a particular inch or a $\frac{2}{3}$-in. may not be that required by others of similar focal length *made by different firms.* We know, for example, an inch that works almost as near as *a* $\frac{1}{3}$*-in.* by another maker. The beginner then should have a care in this matter.

When using a $\frac{1}{3}$-in., which has usually a N.A. varying from ·65 (Zeiss's apochromatic) to, say, ·40, a different condenser must be employed. It is best to use it with an aperture as near as possible to 1·0,[1] for it will then be of service with objectives of shorter focal length than the $\frac{1}{3}$-in., and which have usually a higher aperture still.

Centring the Condenser.—In using a condenser of this description a fresh operation has to be conducted before attempting to look at a specimen; it is called "centring the condenser." This means that the optical axis of the illuminator shall be placed in adjustment or in line with that of the objective. To do this properly must be the aim of the microscopist on all occasions, for without so doing the objective *never performs properly*, especially if it be of high power. It is effected in the following manner, by employing the adjusting-screws provided either in the condenser mount itself or in the substage fitting. To learn the art, anyhow at first, it is best to use no specimen at all, but to rack the condenser up to the level of the stage

[1] It is sometimes a convenience, however, because of the *larger image of the illuminant*, to use a condenser of *longer* focal length and of N.A. ·45 for objectives not above this numerical aperture. We often employ with great advantage an illuminator by the same makers as just mentioned, of exactly this aperture, but condensers of excellent performance are sold by most of the leading opticians. The special care necessary when using a condenser of larger aperture than the objective, lest the image should be "flooded," is discussed in the chapter devoted to the Use and Abuse of the Substage Diaphragm.

and to lower the objective down until it nearly touches it. The correct position is found when the image of the illuminant is in focus. The iris diaphragm should now be closed to a large pin-hole (with the highest powers it must be shut as *close as possible*) and the eye placed at the ocular, a low-power objective, such as the inch in this instance, being selected. Raising the objective, unless the condenser is greatly out of centring, should quickly reveal the small opening of the iris brilliantly illuminated, provided the mirror has been "set" before commencing operations. Sometimes, however, after closing the iris all is dark, and no matter how much the objective is raised or lowered the little opening of the iris fails to be visible. This means that the condenser is wildly out of centrality. Let the objective be returned at once till it nearly touches the front lens of the condenser, and then be very slowly re-raised with one hand, whilst the other slowly opens the iris. A time will come when the lens will roughly focus an edge of the iris at some part or other as it appears stretched across the field of view. At that moment let the objective be left alone and attention be directed to watching the iris as it opens across the field. It will very soon be seen the way it is moving, which serves to indicate the adjusting-screws that must be employed to bring it apparently in the centre. Working slowly and circumspectly, the little opening can be gradually brought nearer and nearer the centre of the field until at last it is centrally placed, when the adjusting-screws should be left. The iris should now open concentrically with the area of the back lens of the objective as seen down the tube when the ocular is removed, but if not, it requires a little re-adjustment until the desired effect is produced. (If this proceeding fails, as it may when an objective of too high a power is being employed; one of longer focal length should be substituted, changing again to the high-power later on.) The process should be repeated over and over again, the illuminator being purposely shifted eccentrically for the purpose of putting it into the centre again, simply for the sake of practice. At length the student will attain such a familiarity with the process and find it so easy that the adjustment will become as easy as focussing and can be carried out at once with a high power, doing away with the use of the low power altogether.

Having learnt then to centre the condenser, it should be

racked up and down until the image of the edge of the lamp-flame be seen stretching from the top to the bottom of the field, the flat surface of the mirror being the best now to employ. The specimen should then be placed on the stage and focussed. If the image of the edge of the flame be not quite sharp now, a little further adjustment of the substage, up or down, will rapidly make it so.[1]

This process is called obtaining "critical light." It really consists in using light that is focussed upon the specimen, as witnessed by the image of the flame, *at the same time that the objective focusses the object to the eye.* If the flame image be considered objectionable, just a touch of the substage screw puts the condenser a shade lower or a shade higher, which spreads out the flame a little more evenly over the entire field; this does not with low powers affect the definition. If it does so seriously as to be noticeable, critical light must be re-obtained and a bull's-eye placed between the mirror and the illuminant. Great care is here necessary, that in doing this the very thing wished to be avoided is not unexpectedly introduced—we mean a spoiling of the definition. Adjustments, however, of both condensers will usually rectify the fault.[2] We confess at times to have found the bull's-eye more trouble than it was worth, save with quite low powers and a very dense object that required all the light possible. When using a Nernst or other electric light or a Welsbach gas lamp the ground glass is used for focussing and obtaining critical light in the same manner as the edge of the lamp-flame is with the ordinary lamp, or a pencil held against the glass serves equally well as the object to focus. Some prefer this plan to any other.

[1] To do this with greater ease, some substages are provided with a fine motion to the condenser. We used to hold this a most useful addition. Of later years, however, especially since it has been shown that there is a certain freedom in focussing the light of the illuminant, and that the advantages of critical light are not restricted to so very refined a position of the condenser as hitherto believed, we have ceased to recommend this somewhat expensive addition.

[2] It should be noted that with quite low powers, if definition be not quite as good as expected, it is a good plan, notwithstanding the use of a condenser, to try the effect of changing the mirror from the "flat" to the "curved" side, or *vice versâ*. For reasons not immediately apparent, but really depending upon nothing else than the alteration in the angle the rays impinge on the condenser, a great improvement is sometimes effected by this change.

When using a $\frac{1}{4}$-in. or a $\frac{1}{6}$-in., having a N.A. of about ·9 or less, no different treatment is required, save that all the care in making the adjustments must be increased, because of their greater magnifying power and because both objectives work so much nearer the cover-glass. Even with these lenses they can with a little patience be lowered so as almost to touch the cover, and focussed afterwards by being *raised* rather than lowered until the student gets accustomed to their use. So, too, with the dry $\frac{1}{8}$-in. or $\frac{1}{12}$-in., but the last lens is one somewhat difficult to use as a dry lens, for it usually works so exceedingly near the cover-glass.

This brings the microscopist face to face with another difficulty not hitherto mentioned; it is that, when dealing with these high powers from $\frac{1}{4}$-in. upwards, to obtain perfect definition the actual thickness of the cover-glass itself has to be taken into consideration. It is for two reasons. First, because if very thick indeed there may not be room enough to bring the front lens sufficiently near to focus the specimen, which of course puts a stop to everything; and the second, because the varying thickness of the actual glass introduces errors in the adjustment of the components of the lens system. Opticians usually correct their lenses to work with a special thickness of cover; that mostly chosen is ·17 mm. or thereabouts, the variation being between, say, ·16 to ·18 mm.[1] If now a cover be employed of, say, ·22, a source of error becomes present which has to be dealt with if the best results are desired. There are two means: one, which we personally prefer—although it adds sensibly to the cost of the objective—is for the combination to have what is called "a correction collar," by the turning of which the required adjustment of the lens to suit the special cover can be effected; whilst the other is to push in or pull out the draw-tube containing the ocular by different little increments until the best effect is produced—remembering, if the cover be *too thick* pushing in is the remedy; whilst if *too thin* (rarely the case), pulling out obtains the correct adjustment. Both of these methods of correction require practice, because the proof of having correctly adjusted for any special cover, after all, only lies in the eye being sufficiently trained *to recognise* when the

[1] Zeiss marks each dry high-power lens with the thickness that is most suitable for the objective in question. It is in *very small* figures on the mount.

finest definition is obtained, and of its ability to judge whether a pull out or a push in, or a turn of the collar in one direction or another, *improves* the image or makes it *worse*! The Abbe test-plate is the best thing to practise on, and over and over again must it be done until a real proficiency be gained; this, however, has been referred to before. But we have said that the thickness of the cover may be so great as to prevent the objective being lowered sufficiently to focus when the combination has a *very* short working distance. For this reason, and to guard against an accident, the student, after having lowered his objective by means of the coarse adjustment as far as he dares, should never finally focus with it when using these high powers, but always employ the fine adjustment instead. It is obvious that by doing this the objective is lowered very gently and gradually indeed; hence, should it touch the cover—as it certainly will if it be a high power and the cover-glass abnormally thick, as frequently found in slides made many years ago—it will do so gently, provided the screw be turned slowly.

The microscopist can tell when contact between cover and front is made, because he will find somewhat suddenly that the fine-adjustment screw has become quite "slack," as if indeed it had in a moment lost all its power. Directly this occurs, without a moment's hesitation, the screw should be reversed, lest the weight of the tube and objective on the cover causes injury.[1]

As we have already pointed out, directly the focus is obtained the correction collar should be used to get the finest definition with the cover-glass in question; but a word of caution yet remains to be stated when adjustment for cover-glass thickness is arrived at by pushing in the draw-tube instead. It is simply this, to see that the inner tube—the draw-tube, as it is called—works smoothly and easily in the outer one, and that the rack-work of the latter runs stiffly; for, if it be loose, the downward pressure applied to the draw-tube may become communicated to the tube itself, and crash comes the objective upon the cover-glass with, perhaps, most disastrous results. The moral of this is either to see that the outer tube is sufficiently tightly held in its rack and that the rack is sufficiently stiff in itself, or to hold the milled head of the coarse adjustment with the left hand, so

[1] Some continental firms have introduced arrangements for preventing accidents of this nature; reliance is not to be placed upon them, for we have seen fractures occur.

that it shall not turn whilst pushing in the draw-tube is carried on with the right.

Finding the Specimen is always more difficult with high powers than with low ones,[1] so much so that it is better by far for the beginner to use a $\frac{1}{3}$-in. to find the specimen and to centre the condenser with, and then to obtain critical light, *changing to the higher power when all the adjustments are perfectly complete.* But the learner will very soon find—perhaps to his sorrow—that yet another trouble will begin to appear with which he has to deal. Changing the objectives is quickly performed, but he may be very disappointed to find that, in so doing, the part of the specimen he has placed so carefully into the field with the low power—say the $\frac{1}{3}$-in.—utterly refuses to appear in that of the high one. This arises because the centring of the two objectives may not be quite similar. It is especially found to be the case when using what is called a "revolving nosepiece." Further, in addition, from the same cause, the arrangement of his mirror, the centring of his condenser, and the adjustment for obtaining critical light are all apparently upset and of considerably less use. If this be so when using a $\frac{1}{6}$-in. or $\frac{1}{8}$-in. what will it be when the change is made to $\frac{1}{12}$-in.? Especially, as we have just said, are these troubles found when using a revolving nosepiece,

[1] In some cases, when the object is single and small as a diatom, very considerable difficulty may be experienced in finding it if anything like a high power be in use. The following method is exceedingly useful and quite simple to employ, and may very frequently save the trouble of changing the objective for one of longer focal length: Most of these little objects have a ring of varnish around the cover-glass, cementing it on to the slip, the material being attenuated and thin at the edge abutting the specimen, and this forms a suitable object to primarily focus upon. The whole of the ring of varnish, however, is too large in diameter to be entirely contained in the field of view at one and the same moment, so a portion only is usually visible. This takes the form of an arc of a circle, and should be shifted about by moving the slip on the stage until it is so placed as to be accurately bisected by an *imaginary horizontal diameter* of the field of view. When this bisection is accurately effected, it is evident—seeing that the object is usually placed in the centre of the area enclosed by the ring of varnish—that if the slide be now simply moved *horizontally*, the object will fall into the field of view without further trouble. If the object be not surrounded by a ring of varnish, the same process can be carried out to find it, by employing the edge of the cover instead of that of the varnish. Finding a specimen by means of the verniers is described later on in the chapter upon Verniers and their Uses.

for it never is, and never can be, made to work truly, for if true with one objective it will not necessarily be so with another. For this reason the firm of Zeiss, with their characteristic desire of perfecting all details, have designed their arrangement of "objective-changers" upon a novel system, and very effective they are we can testify after using them for many years. Their use is described a little later.

All the high powers—say the $\frac{1}{8}$-in. and $\frac{1}{12}$-in.—to be of much real service for resolving purposes, must possess a higher aperture than it is possible for them to be made with as *dry* lenses, and for this reason are mostly constructed as *immersion systems*. In the article on numerical aperture the subject of the immersion lens has been fully gone into and explained, and the object of the construction discussed in all its bearings, so nothing further upon that score need be here said. But there is one point to which no reference was made, as it was left to consideration in this chapter. Seeing the design of immersion systems is for the purpose of admitting more light, or rather light occupying a cone of greater angle, it can be easily understood that the power of such an objective cannot be completely utilised unless the condenser employed with it be a combination equally well constructed to transmit a cone of similar dimensions. In other words, the condenser must have approximately, anyhow, the same numerical aperture as the objective—that is, if the latter is to work to the best advantage. Not only is this true, but the condenser so constructed must be oiled to the slip with objectives over N.A. 1·0 for the same reason as the front lens of the objective is oiled to the cover-glass, viz. to have what is called "optical continuity." Then too, as a matter of fact, the specimen itself must be prepared in a fluid or medium *at least* of the same refractive index as the numerical aperture of the objective, although experience has taught and very fully shown that better definition still, and greater contrast and depth of focus too, is brought about by employing a medium of a much higher index of refraction than the one named. It will be gathered then that the microscopical student has yet something more to learn, namely, how to use the immersion system of lenses. As this is nowadays such a matter of consequence, the subject must be fully dealt with in all its bearings.

The centring of the N.A. 1·30 *substage condenser* can be pri-

marily obtained with the inch, or better for this purpose with the $\frac{1}{3}$-in., objective in the ordinary manner already explained, remembering to raise it up to the level of the stage before commencing operations, and the mirror subsequently set to its approximately correct angle, the $\frac{1}{12}$-in. not being substituted until these two adjustments are complete.

When the change is made, and it is required to see that the condenser is quite central, no oiling of the objective to the front lens of the condenser is required. Even with this high power, for the purpose in view, it can usually be lowered sufficiently near the condenser, so that focussing of the closed iris can be carried out by raising the tube rather than by lowering it. If this raising method for any reason be objected to, the fine adjustment should be used lest the coarse should do the work too hurriedly, or bring the front lens of the objective in contact with that of the condenser. When the closed iris is seen, final perfection in the adjustments can be carried out. Everything so far complete the tube is raised, and the specimen, previously oiled, is laid on to the stage in such a manner that the oil makes optical continuity between it and the condenser.

To "perfect the getting" of critical light with the oiled condenser, we strongly advise the beginner first to employ the $\frac{1}{3}$-in. This we urge because on raising the condenser should it be necessary, if it be overdone and the top lens strike the slip, this in its turn will cause the cover to strike the front lens of the $\frac{1}{12}$-in., which is an accident to be strenuously avoided. It is needless to point out, if the over-raising of the condenser accidentally occurred when using the $\frac{1}{3}$-in., no such accident would take place, because its front lens would be too far away.

Fortunately for those who do not or will not adopt precautionary measures, when the arrangements have been carried out in the order and manner suggested, seeing that *the condenser was raised to the level of the stage to commence with*, it in general only requires lowering instead of any attempt at raising. In executing this downward motion, however, yet another trouble may unexpectedly confront the beginner. It is this. The condenser may have been computed to be used with a rather *thick* slip, and perhaps the slide in use may just happen to be an exceptionally *thin* one, in which case it will be found that as the flame image appears focussed by racking down the con-

denser (*the specimen being in the focus of the objective*) the oil quits the under-surface of the slip entirely, leaving only a vacuity instead.

To remedy this annoying difficulty one, two, or more cover-glasses oiled together should be placed on the top of the condenser, oil being further used to make a complete optical continuity between the covers, the slip, and the condenser. Occasionally a trouble of quite an opposite character presents itself. The condenser cannot be racked up high enough to make a focus of the flame at all. Even when it actually touches the under-surface of the slip, owing to the latter being *abnormally thick*, the condenser requires lifting up even higher.

The microscopist should now try the expedient of bringing the lamp *as near as possible to the mirror*. If this does not succeed, there is no other alternative we know of save that of his having a new front to his condenser that is especially designed to work through a thicker slip. We have three of such to work with different-sized slips, and have often found them of the greatest service. Care should be taken of these little "top lenses" that they are not scratched when put away out of use.

To proceed, let it now be presumed the flame image has been focussed in the field of view by lowering or raising the condenser, then critical light is said to have been obtained.

The $\frac{1}{3}$-in. is now removed and the $\frac{1}{12}$-in. substituted. This lens has now to be oiled to the cover-glass, an operation which is performed by one or two methods in the following manner: Either a drop of cedar-oil is placed on to the front lens of the combination (care being taken not to scratch it) before affixing it to the instrument, or a drop placed on the cover instead, where it is known the objective will come in contact with it. In lowering the tube great precaution and some little practice are necessary.

The coarse adjustment can be employed until the oil makes contact between the front lens and the cover-glass—a fact that will be easily recognised by a little flash of light quite visible when looking sideways at the specimen; but further lowering to obtain the focus *must be entirely effected by the use of the fine adjustment only*, and very gently and very slowly indeed. It should be mentioned (when oil is put on to the cover and the

immersion lens is *lowered into it*) that occasionally the microscopist may *think* the objective is in contact with the oil, when in reality it is not so. Whilst looking through the ocular such is at once known to have taken place by the field of view suddenly becoming *sensibly brighter* as the slow motion lowers the objective and contact is made. If the cover be known to be over-thick, still further caution should be taken, the eye watching through the ocular the first dawn of any signs of the object coming into view, the screw being instantly stopped the moment it seems to become slack, which indicates the front is resting on the cover. Instant reversal then of the screw is necessary, as before explained, to save a crash.

Objectives of N.A. 1·4, having fronts that are hyper-hemispherical, are much more delicate than those with N.A. 1·3 (see page 77, Fig. 77); hence the judicious beginner will commence first with the latter combination rather than the former, because the fronts, being not so hyper-hemispherical, bear rough usage (comparatively speaking, of course) much better than those working at a higher aperture. Final adjustments must then be carried out to obtain critical light, the object and the flame being both required to be simultaneously in focus.

In shifting the specimen about, to examine different portions of it, focussing should be re-made at every short increment of movement, in case the cover-glass is much thicker (or even in some cases slightly bent) in some parts than in others. The microscopist who lets his slide "run" without re-adjusting the focus may break the cover almost before he is conscious of any jamming of the specimen.

From what has been said it will be seen that the whole process of using the $\frac{1}{12}$-in. or other high-power immersion system is one not to be learnt in a minute, neither is it one that can be undertaken without a certain amount of care until continued familiarity renders it comparatively easy; but it should always be recollected familiarity *must never lead to contempt*. Further it will be readily understood that when using a $\frac{1}{3}$-in. (for example) to primarily focus with, to set the mirror with, and so on, *the* $\frac{1}{12}$*-in. should have the same alignment of axis as the* $\frac{1}{3}$*-in.*, for otherwise the object may not be in the field when the high power is substituted for the low one.

This often constitutes a sensible difficulty in carrying out all the

details we have suggested, but to attain to it, and in fact to remove this source of trouble, we have already mentioned Carl Zeiss have invented their sliding objective-changer—a simple arrangement which answers the purpose most excellently (see Fig. 139).

To prepare a battery of objectives requires a little consideration. Before commencing to do so we should mention that the slides consist of two portions—the *tube*-slide and the *objective*-slide—the former (*a*, Fig. 139) being permanently screwed to the nose-

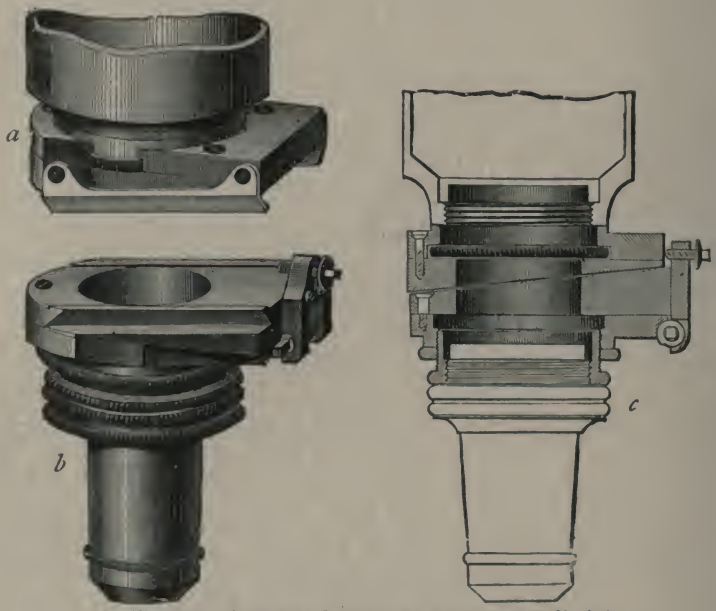


Fig. 139.—SLIDING OBJECTIVE-CHANGER (*full size*).
a, tube-slide; *b*, objective-slide with objective attached; *c*, in section.

piece, and one of the latter (*b*, Fig. 139) permanently attached to each objective.

As we have said, a little care is needed so that all the battery shall be in perfect alignment. To do this effectively the following plan is easily learnt and is thoroughly effective. A low-power ocular being placed in the draw-tube, the highest-power lens of the battery—say an immersion $\frac{1}{12}$-in.—is first screwed on to the nosepiece of the instrument in the ordinary way—that is, *without* the intervention of an adapter or fitting of any kind.

A large and well-defined circular diatom, such as the one we usually use ourselves for the purpose—the Aulacodiscus Brunii—is then placed on the stage, and fidgeted about until it is in the centre of the field and roughly in focus. No oil need be used between the objective and cover, or between the condenser and slip, as the best definition is not required just to place the diatom as nearly central as possible. It is advisable to close the sub-stage iris a sensible amount, otherwise the specimen may be missed, being hidden by the fluffy state of the definition with full aperture and no oil contact. The lens is now unscrewed and laid aside, care being taken in doing this not to touch either the specimen or the stage. The *tube*-slide of the apparatus—that which, as its name implies, is for the purpose of affixing to the tube—is attached thereto in such a manner that the opening to receive the *objective*-slide is in the most convenient position. The *objective*-slide is now screwed on to the objective itself, and the two slides united by slipping one into the other. When the diatom is focussed once more, it will probably be found to be no longer occupying the centre of the field. To make it do so the little watch-key supplied with the apparatus is applied to one or both of the little screws shown in *b*, Fig. 139, forming part of the objective-slide, and a few turns or portions of a turn are made until the desired effect is produced. The objective should be slid off and on again several times until it is made quite certain that the object always appears in the desired situation. It is well now just to understand the rationale of what has taken place. The idea of putting the objective on to the instrument without adapter of any kind was to place the diatom actually in the optical axis of the objective. After the sliders were united the subsequent adjustments with the little screws and watch-key were merely carried out to restore the lens to its original position. This ensures its best possible performance.

The lower powers (each in their own adapters) should now be centred one after the other on the diatom, which is easily effected by screwing an objective slide on to each and adjusting with the watch-key in succession. It is needless to observe *the diatom must not be touched, or the stage interfered with* in any manner during the entire operations. It is very evident now that any object placed centrally in the field with the *low power*

will be central when it is changed to the *higher one*, hence it can be understood all the previous directions of different kinds we have given are much more easily carried out and with far greater precision than if no changers were in use. Moreover, in the everyday use of the instrument it is a great saving of time to find that in changing from one objective to another all centring of the condenser and adjustment of the mirror suitable for the first combination, shall not be immediately upset and require readjustment when employing the second!

The convenience afforded by the accuracy of these objective-changers is manifest in more ways than one. If supposing two or three objectives of the same focal length have to be compared, it is essential, to do the thing fairly, that the same part of the specimen (and consequently the same refinement of the illumination) should be employed with each. Now this can only be done expeditiously in the manner just related, taking one as the standard and setting the others in alignment with it. If it here be said they can be as well tried, one against the other, by first screwing one on to the nosepiece and then the other, we shall admit that the argument is true, *but only if the centring of each lens is exactly similar.* But this is sometimes not the case, and the consequence is that the same, *exactly the same*, portion of the specimen is not in the field of view on each occasion. The secondary consequence of this is that when this original position is obtained, the light will now be found not to be *exactly* central, and all the adjustments have to be made over again to compare one combination fairly with the others. This trouble is prevented by first aligning the different objectives to the same centre as we have suggested. Of course, if one particular combination requires a very large change of position to be effected by the little adjusting-screws before it is centred to the same alignment as the rest, it is possibly best to examine that one separately, by placing it directly on to the nosepiece; but this does not often happen.

Then the great comfort afforded by the use of these changers is manifest in another way, namely by the ease with which a specimen may be run over with a low power, any spot requiring the aid of the more powerful combination being readily examined simply by the interchange of objectives, no adjustments of any kind being further required save that of altering the focus.

Similar ease of examination can rarely be obtained with the revolving nosepiece, as no means is provided for setting objectives in one and the same alignment. We are aware that some opticians assure us that their nosepieces are perfect in this way, asserting that they are made so exactly that one objective will follow on the other quite truly; but surely they forget that the fault mostly occurs in the actual mounting of the combination itself rather than in the nosepiece, which no amount of accurate manufacture can possibly correct unless some special means of adjustment is provided.

When there are only *low* powers in the battery, as obtains with many amateurs, this exact centring of each objective is not of such primary importance; indeed, the refinement is almost uncalled for in most cases. Under these conditions the objective changers of Zeiss may be found a trifle cumbersome,

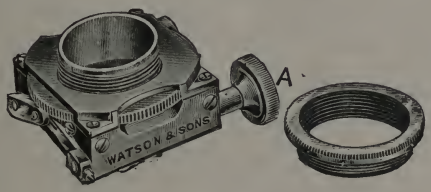


Fig. 140.

and perhaps too costly, although lately reduced in price. To offer a better means of rapidly changing the different powers other than by the rotating nosepiece, which although commonly in use has the disadvantage of weighing heavily on the tube, especially when filled with three, if not four objectives, Messrs. Watson & Sons have introduced an exceedingly efficacious changer made in magnalium. This is fixed to the nosepiece, and is so constructed as to grip the screw end of the objective—protected by a little ring, sold for a few pence, to attach to each combination—so firmly and so truly that it would seem to leave nothing to be desired. It is called the "Facility" Objective-changer, and is shown in Fig. 140. We have used one for some time on a low-power instrument, and it has given us every satisfaction. Its size, however, is not suitable to the Continental model of microscope, as it interferes with the movement of the tube in its slides. To use it is very simple. The turning of the handle A causes the inversion downwards of a pair of jaws having a

screw-thread cut upon them. The objective is placed in the aperture, and directly the handle A is released the jaws are carried back to their natural position by the action of a boxed spring, in doing which their threads engage the threads of the objective and carry it up to the shoulder. In consequence of the varieties of sizes, within very small limits, that still prevail with objective threads, it has been found desirable to supply the rings mentioned to screw to the objectives, these having threads of an absolute gauge which will be gripped by the jaws of the nosepiece with certainty.

Before quitting this part of our subject a few words may be said with respect to the way of using the eyes. Of course, only one can be employed with the ordinary instrument at a time, and what follows refers more especially as to what to do with the other. This should never be closed up tightly by its own muscles, as a strain thereby effected is very apt to spoil the

Fig. 141.

perceptive faculty of the eye in use. Neither should the hand be used to shut it. It is best to learn to keep it open; but if this cannot be acquired, the hand should only *shade*, and not touch or close the eye off duty, so that, although it is prevented seeing anything, it is not kept *shut*. It is best, as we have said, to keep it open; but when doing so the observer, in addition, should try to make it turn *as if it were looking at the object seen by the eye in use*. By this means an unconscious similarity in the focussing is brought about at one and the same moment. If, however, the observer lets his "off-duty" eye simply become a blank, then this focussing is not effected, and it may be that a certain kind of diplopia (double sight) may be brought about by this means, which is very unpleasant; for the effect is that the observer, after leaving the microscope, apparently sees double, even, perhaps, for an hour afterwards. For those unable to learn to do what we have said, a neat little arrangement is made to fix on to the draw-tube that holds a

small shield, effectually cutting off "the seeing" of the eye not in use. Some users of the microscope regularly employ this ingenious little piece of apparatus. It consists of two parts, as shown in Fig. 141, jointed in the middle; one part has in it a circular aperture, which slides over the draw-tube of the microscope, and the other shuts out light from the disengaged eye. It is usually made in vulcanite, in two sizes, one for the Continental, and the other for the English size draw-tube.

The Position the Verniers should Occupy and the Method of Centring a Circular Stage

If a 3 × 1-in. ordinary glass slip be taken and its centre marked with a dot of ink, this dot should be exactly $1\frac{1}{2}$ in. from either end and $\frac{1}{2}$ in. from either side. This may be called the centre of the slip. If a cover-glass be taken with a circular diatom, such as an Aulacodiscus Brunii burnt on to it, and the diatom placed so as to cover the dot, then the diatom will show the centre of the slip in a more convenient fashion. All these measures need only be approximately correct.

Placing this "centring slip" upon a square-shaped movable stage and by the use of the ordinary milled-headed screws in such a position that the diatom occupies the centre of the field of view when using a $\frac{1}{2}$-in. (or other low power) and a × 4 ocular, it will be found, if the verniers are fairly well located, their readings will be about midway of their length. For example, if one be 60 mm. long and the other 40 mm., then the readings should be approximately $\frac{30{\cdot}00}{20{\cdot}00}$. If they differ very much from these figures, supposing they were $\frac{40}{30}$, for example, the instrument should be returned to the makers, because when used with a slip having *two* covers upon it, in all probability the verniers will not be able to be used, as one of the covers, if not both, will be beyond the reach of the graduations. By this we mean the scale readings will be at an end before the stage is shifted far enough. Providing the verniers are reasonably well placed, a high power should now be used instead of the low one and the exact readings of both verniers taken when the diatom is exactly in the centre of the field, a note being

made on the centring slide, with some additional indication as to which way it is in the future to be placed on the stage. A capital letter R on the top right-hand corner is the method we employ ourselves.

If now at any time in the future, after having set the vernier to the previously recorded position marked on any particular slide, the special position of interest desired to be seen fails to appear in the field of view—provided, of course, such positions were correctly recorded—it must arise from the centrality of the stage having by some means been upset, or, what is the same thing, the verniers must have accidentally slipped. To enquire into the fault the "centring slide" should be placed on the stage in its proper position as recorded upon it. If no accident has occurred to the stage of course the aulacodiscus should appear in the centre of the field, in which case the previous "failing to appear" evidently has arisen from some mistake in the notation *upon the slide* in question; but if, on the contrary, the centring diatom does not come into the centre of the field, it proves most unmistakably that some accident or other has upset the bearings of the stage. To set matters right, the aulacodiscus should first be made central in the field, according to its recorded figures, by means of the ordinary stage screws, after which the verniers themselves should be released from their bearings by undoing the minute screws that retain them *in situ*. A slight enlargement of the little holes through which the minute screws pass will enable the graduations to be set correct again, so that the verniers once more read according to the figures noted on the "centring slide." This done, the recorded positions of interest on slides containing specimens will now once again be correct.

With a circular revolving stage, however, an additional motion has to be taken into consideration; it is that whilst revolving around the optical axis *the specimen shall remain in the centre of the field.* Hence with a stand of this description, directly it comes from the maker the centring slide should be placed on the stage and adjusted to the centre of the field by the ordinary milled-headed screws, first with the low power and then with the highest of the battery, *taking care there is no changer or revolving nosepiece used at all.* Revolution about the axis is then made and the diatom watched. If no "travel" of the diatom

can be noticed about the field—merely its own revolution on its axis—then the axis of revolution of the stage *is* concentric with the optical axis ; but if, on the contrary, it appears to describe a crescent-shaped path, as shown in Fig. 142, it proves that the axis of the stage is not in alignment with that of the objective. To make it so, one or more of the little screws usually provided for the purpose in all well-made stands have to be turned, but the question of difficulty always is to decide which. To discover a solution of the difficulty is quite easy if the following plan be adopted: The eye must watch the arc described by the diatom before referred to, and this should be mentally

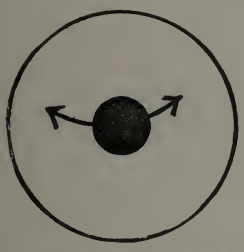


Fig. 142.

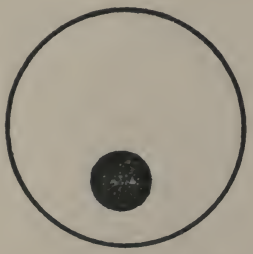
Fig. 143.

compressed into an arrow-head, the apex of which will point directly to the edge of the field *to which the diatom requires moving*, as indicated in Fig. 143.

Each screw can be now gently tried in succession until the right one be found, or it may even be discovered that the direction requires the use of two. The diatom need not be moved far at first, but left in the position shown in Fig. 144. It is now recentred in the field, as in Fig. 145, by the *ordinary* stage screws and the stage revolved again. If it still "travels," then another shift must be made, and so on, again and again, until at length no movement is detected, save of course its own revolution on its axis. It should be recollected in securing

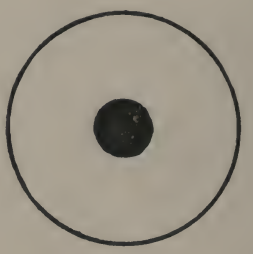
the adjustment, through *one* screw being not turned enough, or one of the two, if two were used, being more turned than the other, *the situation of the arc in the field* may frequently change its position, but this requires no alteration in the method of treatment. The arrow-head must be mentally pictured each time and a fresh adjustment made.

When as perfect as can be, a higher power should be used, and finally the highest of the battery. It should be mentioned that with a great magnification the motion will not appear smooth and regular except in the finest instruments, and a certain allowance must even then be made for the unexpected



AMOUNT OF SHIFT TO BE TRIED AT FIRST BY MEANS OF THE SPECIAL ADJUSTING SCREWS

Fig. 144.



DIATOM PLACED AGAIN IN CENTRE OF FIELD BY THE ORDINARY STAGE SCREWS

Fig 145.

presence of exceedingly small pieces of foreign matter—thickened grease—or the like making it apparently jump a little until they are removed.

In numbering the slides for the cabinet, room should always be made for the vernier readings, and, as we have suggested, the letter R should always be placed on the top right-hand corner of each slip to indicate its position on the stage ; and it is convenient, we have pointed out before, *always to put the reading of one vernier* at the top and that of the other underneath, so as to be able to differentiate the verniers to which they apply. Thus $\frac{30{\cdot}45}{25{\cdot}10}$ means the vernier moving the stage up and down must be placed at 30·45, whilst that recording

horizontal motion is to be set at 25·10. We have repeated this suggestion because many microscopists in using their verniers in an irregular manner—one reading of the vernier being uppermost in one case and just the reverse in another—have led them to think mechanical recording stages to be of no service. So too with placing the R always at the top right-hand corner: if this be not always done, it is impossible to know at some future date which way the slide has to be placed on the stage.

Reading the Verniers.—The ingenious arrangement known by the name of a Vernier was the invention of a French mathematician, Peter Vernier, who flourished somewhere about 1630. In the microscope stage there are usually two, one being to record the motion up and down as seen in the field of view, the other to indicate a movement from side to side.[1] Each consists of two scales, a long one attached to some *immovable* portion of the framework, and a short one affixed on the contrary to some part that is movable—in point of fact to one of the frames that hold the specimen. The smaller is so arranged that it slides along the edge of the greater scale, and both should be within easy sight of the microscopist.

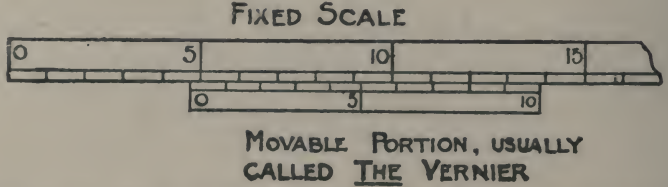
The longer scale, usually about 50 mm. in length, is graduated throughout its entire length, more commonly nowadays in millimetres, whilst the smaller or movable portion has divisions engraved upon it only for a distance equal to 9 mm.; *but this space is divided into ten equal divisions.* It is obvious then that each division of the *smaller* scale (which is mostly called by the simple name of *the* vernier) is exactly one-tenth less than each division of the long one. In this lies the principle of the arrangement.

In order to read a given position of the vernier, we ascertain for certain the scale of 9 mm. *is* divided into ten equal parts, and that the first line of the divisions is marked 0. This is called "the zero" of the vernier. In "taking a position" we first note the position of this zero; let for example it lie as in Fig. 146, between 4 and 5 on the greater scale. We write down 4, because it is evident the position, although greater than 4, is less than 5. We next proceed to find out how much greater this position taken up by the zero really is, and consequently

[1] Sometimes a *third* is added to indicate the amount of revolution of the stage about the optical axis.

how much more we must add to the 4 to make the reading correct.

To do this the eye is run along "the vernier" scale until it reaches a division that will correspond in alignment *exactly* with one on the fixed scale. This we see in Fig. 146 occurs at the eighth division of the scale in question; for there the line appears to be exactly continuous with the twelfth division of the greater scale of millimetres. As this distance at the eighth division of the smaller vernier is equal to eight-tenths of a millimetre (because we have already shown each of the divisions of the smaller scale was one-tenth less than each of the greater), so we learn the amount to be added to the prime figure 4 is simply ·8, which makes the correct reading 4·8. If the reading happened to have



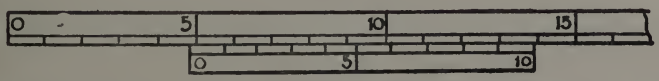
VERNIER SHOWING THE 8TH DIVISION COINCIDENT WITH 12TH AND SO READING 4·8.

Fig. 146.

shown the third division of the vernier scale, instead of the eighth, was in alignment with some other division of the fixed scale, then we should have only added ·3 to the 4: whereas, if the alignment had been at the sixth, then the correct reading would have been 4·6 and so on. Should the zero in the figure have been resting between 0 and 1 of *the free millimetre scale* instead of between 4 and 5, then the figure 0 would have been written in the place of 4; and if the subsequent alignment had been found to have occurred at the first division of one scale with that of the other, making as it were one continuous line, we should have only added ·1, the reading appearing as 0·1.

So far it is easy to understand; but the case may arise—indeed it often does when using the stage verniers of the microscope—that having noted the prime figure, as for example the 4 in the previous argument, we *cannot* subsequently find any

two divisions that are really actually in alignment between the scales as represented in Fig. 147. The vernier reading 8 is there seen to be just as much in advance of the twelfth divisional marking on the fixed scale as the ninth of the movable is behind the thirteenth of the fixed one. Seeing then the advance in question really means half a tenth or $\frac{1}{20}$, or 0·5 millimetre, so we get over the difficulty by adding that amount to the reading, making it finally 4·85. The scales will not readily admit of finer differentiating, although the practised eye may distinguish between ·025, ·05, and ·075 ; but in reality such refinement is never required with the microscope. It should be understood, however, in passing, that of course verniers, when so desired, can be made to read quite conveniently to the third place of decimals of inches, as for example we usually find obtains in such instruments as the standard barometer.



VERNIER SHOWING THE 8TH DIVISION ABOUT EQUAL DISTANCE BEYOND THE 12TH. AS THE 9TH DIVISION IS BEHIND, 13TH READS 4·85.

Fig. 147.

It now remains to be explained how to set the verniers to take up a given reading. For simplicity let it be desired to set them at 4·00. We slide along the vernier until its zero rests at the required figure on the fixed scale, and the thing required is done. Should, however, the numerals be 4·8, it is evident the zero must be passed along beyond the 4, nearer to the 5, because 4·8 is $\frac{8}{10}$ of a millimetre more than 4·00. Accordingly we count eight divisions on the vernier scale (that is to say the smaller one), and, fixing the eye upon the graduation, move the whole stage, which causes the vernier to shift in the required direction until this line meets with the first graduation on the great scale, to which we set it in alignment. This is the line 12 as indicated in Fig. 146. Supposing, however, that the figures we wish the vernier set to are 4·85, we recognise that the vernier scale yet requires more movement still, so that the zero shall be shifted ·05 further. To do this we gently turn the stage screw a trifle,

just enough in fact to make the 8 advance over the twelfth division about as much—that is to say about the same distance—as the ninth division is *behind* the thirteenth (Fig. 147). The object for which the readings are taken, should now lie in the centre of the field of view in the ocular, provided the figures furnished were correctly given.[1]

[1] We should again mention that for the object to appear in the centre of the field of view another thing is necessary. It is that the stage itself shall not have shifted out of centre since the record upon the slide was made. This is the reason that stages of first-class make are provided with centring screws, so that, should the upper plate become eccentric through wear and tear, it can always be *re*-set in alignment with the optic axis. When this is so the readings on the specimen will once more be of service, provided of course the stage was truly central at the time they were taken. It has already been explained how this is effected.

CHAPTER XI

THE BINOCULAR MICROSCOPE AND STEREOSCOPIC VISION

THE ordinary form of this instrument is illustrated in Fig. 148, where by mere inspection it will be seen the leading difference between the Binocular and the Monocular microscope is that the former is provided with two tubes (and two oculars)—one for each eye—whilst the latter is only provided with one.

By this arrangement it is possible to produce—in the manner about to be described—the effect known as "stereoscopic vision," which, explained briefly and simply, according to the theory propounded by Wheatstone, is as follows: Let a thin book, say about an inch in thickness and five in width, be placed upon a table, standing upright thereon as it does in fact upon an ordinary book-shelf. The back of the volume where the title is usually printed must face the observer, and be in an exact line with his nose at a distance of, say, 18 in. therefrom. Each eye will now just be able to see a different side of the book, the right eye the right side, and the left eye the left one. When both eyes are simultaneously employed, the book looks to be very solid and to have a decided *depth*, no confusion arising in the mind of the observer by the blending of these two images in question, although taken from two different standpoints. The same effect of depth is produced in the stereoscope, where two photographs, each from a different point of vantage, are simultaneously viewed through the instrument. Here, in point of fact, the union we speak of produces such a strong and pronounced mental impression and suggestion as to the depth dimension that details—say in the photograph of a landscape—that lie one behind the other appear to do so in such a marked and vivid, distinct and unequivocal manner that no possibility

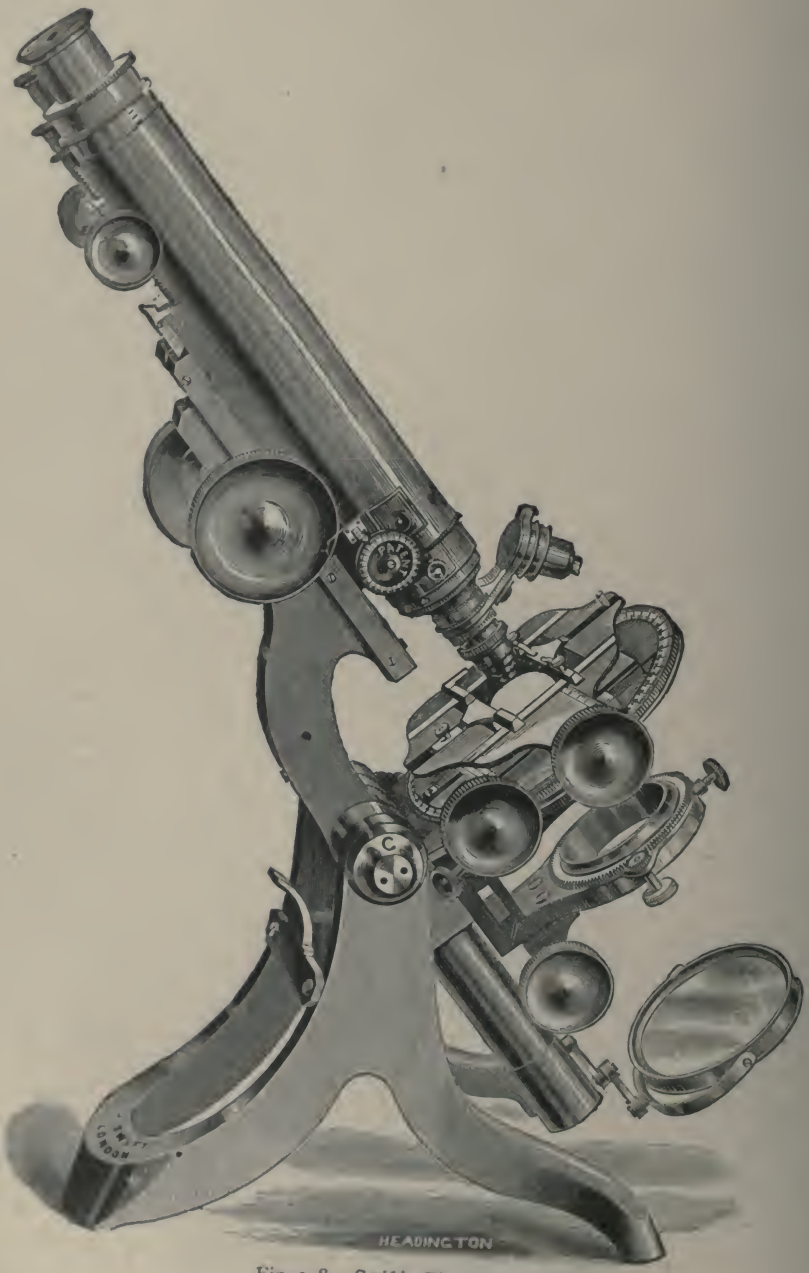
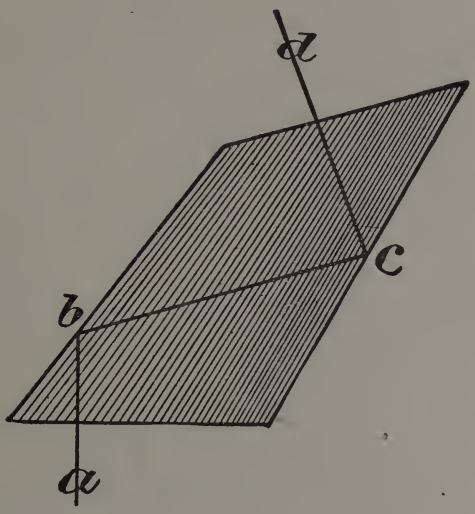


Fig. 148.—Swift's Binocular.

of doubt can rest for a moment in the mind of the observer as to which are really in front and which are behind.

It is the aim then in the Binocular Microscope to imitate this effect—this increase in the recognition of the depth dimension—so that the details of the object on the stage shall show up their relative positions one to the other in the same unequivocal way, those in front being made to "stand out" apparently well above or in front of those that are behind. To bring about this effect a prism is supplied of peculiar construction, named after its



WENHAM'S PRISM

Fig. 149.

inventor, Mr. Wenham, which, when mounted in a suitably constructed carrier, slips into the *body of the tube* just above the nosepiece and below the point of union of the two tubes (see Fig. 148). It is so arranged that it can be withdrawn when not required, and then the right tube being alone in use the microscope becomes once more a simple monocular instrument.

This prism (Fig. 149) divides the cone of rays proceeding upwards from the objective by intercepting the *right* half,

which passes, as shown in the figure as *a*, *b*, *c*, *d*, after two reflections into the body and thence through the *left* tube of the instrument to the corresponding eye of the observer. The *left-hand* half of the cone, however, is not deviated or interfered with in any way, but passes uninterruptedly into the *right* tube and so to the right eye of the observer. But little consideration is sufficient to show that the right-hand half of the cone of rays—those beams proceeding to the *left* eye of the observer—travel a much longer distance than those of the opposite side; hence it is obvious the image formed by them is the more magnified of the two. To correct this inequality of amplification in the images, the magnifying power of the left eyepiece is purposely made to be less than that of the right. It is usual to find the right and left images are not *quite* of equal merit, although they ought to be nearly so. When using both eyes, however, *small* differences are not noticeable. (See Hints, end of Chapter XVIII.)

To adjust for inter-pupilary distance the eyepieces are raised or lowered by means of the milled head (see Fig. 148). This adjustment should be carefully carried out, as some fatigue is caused to the eyes if it be neglected.

The Wenham prism is not suitable in its ordinary form for objectives of shorter focal length than about half an inch, unless specially made for the purpose; but we are informed even then the images leave something to be desired. Neither can the prism be used on the short tube instrument. To meet this difficulty Dr. Greenough suggested, and Zeiss made in pre-war times, quite a different form of binocular. It met with such a good reception that both English and American opticians now make them.

In this arrangement it will be seen by consulting Fig. 150 stereoscopic vision is obtained, not by a division of the pencils of light (one going to the right eye and the other to the left) passing through a *single* objective, but by an ingenious combination of two juxta-positioned microscopes which are complete in themselves, *each having its own objective*. Instead of oculars capable of being raised or lowered to suit the different inter-pupilary distance of different observers as obtains in the usual form (Fig. 148), each microscope has an erecting Porro prism fixed to it, made after the same style as that supplied in the firm's ordinary prismatic binoculars for field purposes now so well known. By rotating these the change for inter-pupilary

distance is readily effected. The microscopes are simultaneously raised or lowered by the use of a single coarse rackwork, a fine adjustment not being required.

The magnification by this arrangement cannot be raised more than about 70 diameters, which in our opinion is quite sufficient for all stereoscopic effects. We should mention certain dia-

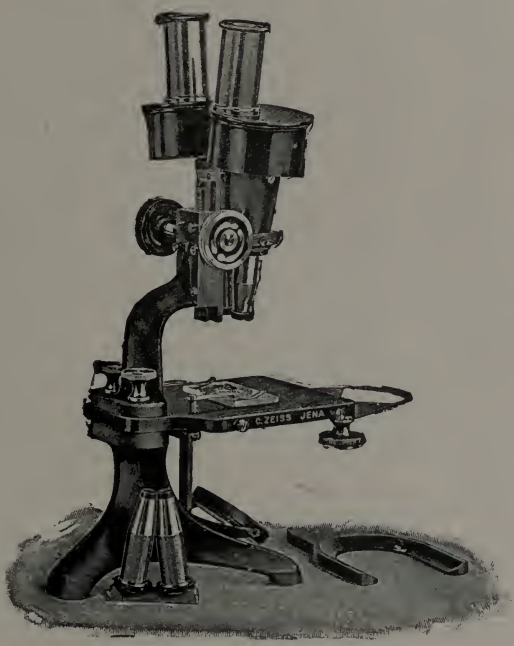


Fig. 150.

phragms are also supplied beneath the stage which assist in perfecting the results.

Seeing that Wenham's prism, as we have already said, is stated not to be suitable for high powers in its usual form, and that some observers are not content with the performance of that form made for objectives of short focal length, Professor Abbe designed a special kind of stereoscopic eyepiece that drops into the *draw-tube* of the monocular microscope, which is itself lowered sufficiently to make the tube-length the correct amount for which the objective is computed.

The eyepiece is shown in Fig. 151.

It will be seen in the illustration that the division of the pencil of rays emerging from the objective—for the purpose of producing two separate images—is effected at the upper end of the tube by partial reflection at a thin stratum of air between the two opposed glass prisms. This air space is now made ·01 mm. thick. One set of rays passes straight through the

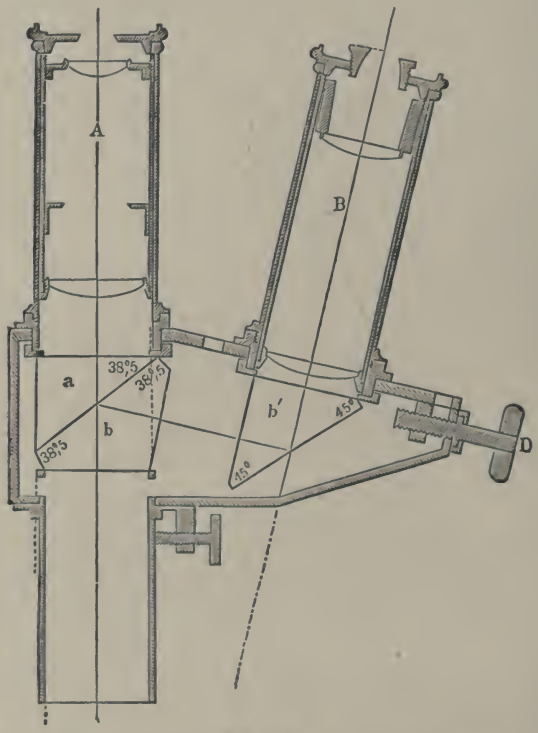


Fig. 151.

Abbe's Stereoscopic Eyepiece (*two-thirds full size*).
(The lenses in the eyepiece B are incorrectly drawn.)

prisms *b* and *a* to form an image in the A ocular, whilst the other reflected on the hypotenuse of the rectangular prism *b′* passes into the ocular B, which is inclined at an angle of 13° to the axis of the microscope. To adjust the eyepieces for inter-ocular distance, the screw D is provided. In order to obtain perfect vision the two oculars must be constructed to give equal definition and magnification, so to fulfil these conditions are made

differently, ocular A being an ordinary Huyghenian of a focal length of 45 mm., whilst ocular B is of the Ramsden form of similar focal length.[1] It is necessary to carry out Professor Abbe's views[2] to cover up portions of each ocular, hence two half-moon diaphragms are provided. These must be arranged so that the *inner* portion of each beam is occulted, consequently for stereoscopic projection the observer only uses the *outer halves* of the rays of each ocular; whilst for pseudoscopic vision[3] the diaphragms are placed in exactly the opposite position, the *inner* halves of the beams being alone employed.

The arrangement when first brought out was not at all well received on account of an objectionable double image in the eyepiece B, which arose from the stratum of air above mentioned being too thick. In the recent instruments, however, by reducing this in thickness, the objection has been entirely removed, and we much regret that recent works upon the microscope have failed to notice this great advance—the entire removal of the objection above stated—as it leads the reader of the present day to have nothing to do with one of the most ingenious and convenient of modern appliances. Through the kindness of the Firm we have had one to examine for a sensible time, and struggled to see the faintest ghost of a second image, but entirely failed to do so, probably because it was not there!

The eyepiece can be used for high powers, but achromats *only*, as compensating oculars for optical reasons cannot be provided.

[1] This is an ingenious way of equalising the magnifying power of the eyepieces, as the focus of the objective lies *outside* the Ramsden but *inside* the Huyghenian. The lenses in Fig. 151 are incorrectly drawn.

[2] Professor Abbe's theory of stereoscopic projection in the microscope (for which the reader should consult his original papers, scattered, it is true, and somewhat difficult to find upon the subject) is not the "view" here referred to at all. This only relates to his *method* of obtaining stereoscopic vision with the microscope, and his departure is merely that, instead of dividing the light *at the back of the objective* itself, he divides it in a very similar manner in the *image* at the back of the eyepiece instead, *i.e.* at the Ramsden circle. Optically speaking the *effect* is absolutely the same as in the usual binocular microscope, where the Wenham prism is used.

[3] If an object like "a jelly," as it ordinarily appears on the table, were under observation, the stereoscopic projection gives to it depth, and it is seen to stand up on the dish; pseudoscopic vision is the reverse, when it appears to the eye sunken and receding, as if indeed the jelly *mould* were being looked at instead.

We should mention, before concluding, that the comfort experienced by certain observers—although not recognised by all—in using both eyes instead of only one, led Messrs. Powell & Lealand to construct a special form of prism which, placed in the Wenham carrier and used in the same manner, converts the binocular microscope into what may be called a "double-visioned" instrument. The peculiarity of this arrangement is that *no stereoscopic effect is present* we are informed, and that it can be employed with any objective whether of short or long focal length. The prism is shown in Fig. 152, and its action is simply this. Part of the light that falls upon the parallel-sided plate of glass passes *through it* to one eye, whilst the portion reflected *off* it passes into the right-angled prism adjacent, being totally reflected to the other.

Fig. 152.

It is said that the stereoscopic effect can be immediately produced when using this prism by merely so arranging the inter-pupilary distance that the *outer* halves of the beams coming from each ocular are used by the eye; and, moreover, that by altering such distance in the opposite direction so as to employ the *inner* portions of each beam, the reverse effect, called "pseudoscopic vision," becomes at once apparent. This, it is said, can equally well be produced by the use of Abbe's diaphragms, of which we have just spoken.

In pre-war times other new binocular arrangements have been devised, but most, at the moment of going to press, are for the time unobtainable.

A very fine entire instrument by Beck is worth attention; and the "Bicor," by Watson & Sons, can be arranged to fit on some existing stands. See Addenda for some interesting information.

CHAPTER XII

MEASURING OBJECTS WITH THE MICROSCOPE AND THE UNIT OF MEASUREMENT USED BY MICROSCOPISTS

It not infrequently happens that it is required for scientific purposes to obtain the exact dimensions of a given object—such as a diatom, for example, or of a minute organism like a bacillus. These objects, being in the microscopist's point of view comparatively large, do not offer so very much difficulty after a certain amount of practice has been attained; but when extremely minute details have to be measured, some of which are even commensurate with the wave-lengths of light—as, for example, the dots in Amphipleura pellucida—the operation taxes the powers of the operator to the utmost extent.

The method of carrying out these measures is by the use of

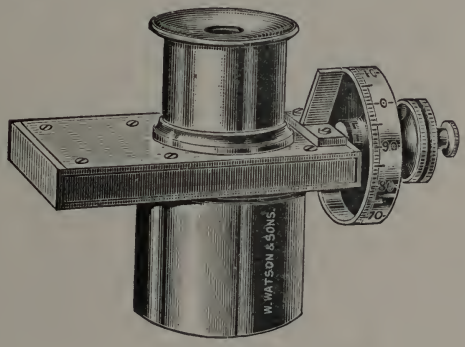


Fig. 153.—Spider-line Micrometer.

an instrument called a Spider-line Micrometer (Fig. 153), which is placed in the draw-tube instead of the ordinary ocular.[1] It may be sufficiently described for the present purpose by saying that such is a contrivance by which two wires are viewed simultaneously with the object to be measured, through lenses which form part

[1] In speaking of this as the instrument usually employed, we are not unmindful of an entirely new and distinctly original arrangement devised by Mr. J. W. Gordon, a full account of which is given in the Addenda.

of the arrangement. One of these wires, although capable of a limited amount of adjustment when necessary, is immovable when a measure is being taken, and hence is spoken of as the "fixed" wire or thread; whilst the other, working in a separate frame, can be moved by a delicate screw, a hundred threads to the inch, across the field. The screw itself ends outside the eyepiece in a divided head, shown in the figure, called the drum, the circumference of which is engraved with exactly a hundred divisions, for the purpose of showing portions of one entire revolution of the screw. To make the webs appear distinctly sharp, the eyepiece containing the lens is capable of being pulled out or pushed in, so that, when the observer looks through the apparatus, he sees the wires distinctly in focus; and, if not so, he proceeds to make them so before putting the arrangement on to the microscope. The actual number of *complete* revolutions of the screw is known by looking *into* the eyepiece, and counting how many notches in a comb stretching across the field of view (and also in focus with the wires) have been passed over by the movable thread.[1] This is the usual form of instrument; but there are many variations in detail which are not mentioned, to avoid any complication arising in the reader's mind, inasmuch as it is only the *principle* of the construction that is really necessary for him to understand at the moment.

A little consideration, and it is readily understood to make a measurement all that is necessary is to arrange the micrometer in such a position that the fixed wire shall lie apparently in contact with one end of the object, whilst the movable one is run across the field so as to enclose the other. A count of the notches of the comb over which this latter thread has travelled in its passage across the field to enclose the object represents the total number of whole revolutions of the screw, whilst the readings on the drum-head furnish the hundredths in excess that have to be subsequently added to complete the measurement. The figures thus obtained are necessarily in terms of the revolution of the screw, so that to make them of practical value they must be reduced into those of the inch, millimetre, or micron, whichever is desired. This conversion implies the

[1] The threads or wires of a micrometer are really constructed of *exceedingly* fine cobwebs. Originally they were made with the finest wires or silk threads procurable; hence the origin of the term in question.

ascertaining of the value of a single revolution of the screw, and is called "orienting or evaluating the micrometer." It is one that obviously must be very carefully executed, for otherwise all measurements taken are practically valueless.

Before commencing, however, it is absolutely necessary for the observer first of all to make sure his fixed thread is correctly placed with respect to the movable one; for it is evident, if this be not so placed, an error in all measurements must take place. To test it, let the drum be set exactly at 0, and the observer look through the ocular. Presuming the wires are in focus, the movable web should be seen *exactly* over the fixed one. If this be not the case, it must be made to take up that position by means of the little *adjusting-screw to the fixed thread*, provided for the purpose outside the apparatus. Having now set the draw-tube to such a length that the ocular is very approximately the exact distance from the shoulder of the objective for which the latter is corrected, a stage micrometer is placed on the stage and illuminated in the ordinary[1] manner. The rulings usually selected, when it is desired to measure the object in English units, are those a ten-thousandth part of an inch apart when employing a twelfth, but of course of greater interval with lower-power objectives. The rulings on glass usually have a somewhat granulated appearance under the microscope, and are mostly wider—that is, broader—when a high power is used than either of the fine spider webs; hence it is necessary to place each web in the *centre* of the line so as to bisect it its entire length. To do this from the upper to the lower end of the field requires a little patience, turning and twisting the micrometer about the optical axis until it is accomplished.

Having by means of the ordinary stage screws apparently placed the fixed wire of the micrometer on the first line of the *stage* micrometer (which is called 0), the movable web is shifted along by means of the micrometer screw until it rests upon the tenth division, care being taken in each case that the wire bisects the line upon which it apparently rests from its upper to its lower end, as explained above. The number of notches in the comb between these threads is then counted and noted. Let this, for

[1] The Stage Micrometer is the name given to a cover-glass ruled with lines a certain distance apart, and mounted in balsam on to an ordinary 3 × 1 in. slip.

example's sake, be supposed to be 14. Then we count the hundredths on the drum (if there be any)—which we may by way of illustration say are 41 in number—and add them to the 14, making a total of 14·41. But if the pointer to the drum shows an excess over the 41, and yet not enough to call the reading 42, this quantity must be then estimated by the eye and finally added to the 14·41. Suppose it is considered as equal to ·5 *of a division*,[1] then ·005 is added to the 14·41, making a grand total of 14·415.

The micrometer should now be moved away at random from the lines on the stage micrometer, and the whole process—beginning with the resetting of the fiducial wire—repeated again at least four times, if possible, over different rulings[2] of the micrometer. Presuming the readings ran thus:—

14·415
14·390
14·550
14·452
14·428
5)72·235
14·447 mean,

then 14·447 represents the mean value of ·001 in. (10 intervals of $\frac{1}{10000}$ in.), and therefore that 1 revolution = ·00006921, or $\frac{1}{14447}$th of an inch. The above figures show that either the rulings were not exactly equal or the observations were not absolutely accurate; *in reality*, however, the errors are not so large as they at first sight appear, for the difference if worked out is exceedingly small.[3]

Without touching the draw-tube the object can now be measured

[1] This of course means $\frac{1}{200}$, or half one of the hundredth divisions.

[2] Sometimes the stage micrometer does not furnish more than ten intervals. In this case change the lines from above downwards or *vice versâ*, any method being adopted to secure a "fresh place."

[3] If it be desired to show with what *degree* of accuracy the measurements have been made, the *absolute values* of the deviations from the mean are added together and *their* mean obtained, which is stated. In the above the "average deviation from the mean" is ± ·043. The reader should consult an interesting paper upon the errors in the rulings of stage micrometers which have been arrived at by Mr. Marshall D. Ewell, President of the American Microscopical Society (1907), after a series of painstaking experiments, *Journal of the Royal Microscopical Society*, Nov. 1908.

and its dimensions in terms of the inch estimated, several settings and estimations being made and their mean taken. In carrying out this measurement, it will be found exceedingly difficult in many cases to know which is *really* the edge of the object, for diffraction phenomena may prevent such being exactly seen; hence an element of doubt always exists in the measurement taken of extremely minute objects. Then too the shake of the tube when using the micrometer makes the act of setting difficult. Mr. Nelson's suggestion to support the micrometer on a separate stand, so that, although in alignment with the optical axis, the micrometer never touches the draw-tube *at all*, is a valuable one. We have found it facilitates obtaining accurate measures, and is one to be cordially recommended.

As we have said before, it is difficult to be certain of the real edge of the object. The following are Mr. Nelson's suggestions :—

(1) Use objectives with as large an aperture as possible with the largest illuminating cone procurable.

(2) Measure from the *inner* edge of the *inner* diffraction band to the *inner* edge of the *inner* diffraction band on the opposite side.

(3) In measuring the diameter of *a hole*, measure from the *outer* edge of the *outer* black diffraction band to the *outer* edge of the *outer* diffraction band on the opposite side.

(4) The focus of the object to be chosen is what may be termed that of the "black dot"; in other words, if the object were a slender filament it would be represented white with black edges. These black edges are due to diffraction. If the filament is very slender and the illuminating cone small, there may be seen a *white* diffraction edge outside the black one, and perhaps another faint black one outside it again.

Although, however, we have quoted in full the suggestion of so eminent an authority, we must reluctantly admit, save with No. 1, we do not agree with his remarks. In making measurements of small objects such as minute holes, bacteria, or flagellæ and the like, we admit it is difficult to estimate the position of the real size of the object, but a little consideration at once shows that this difficulty really arises from "the limit of resolving power" imposed by the undulating theory of light, so any measurements *must* in consequence be uncertain within limits comparable to the resolving power of the objective employed. Hence it seems obvious any attempt to establish hard-and-fast

rules by which the true size of such minute objects may be correctly estimated, is quite useless if not absolutely unscientific, for is it possible to accurately measure an object we admit cannot really be seen? The measurement of the *distance apart* of small discrete objects or markings—such as diatom dots—is, however, another matter altogether, for here theory teaches that, with carefully regulated illumination, the effect of the limited resolving power of the objectives is merely to give to the objects a fluffy edge all around, so that (notwithstanding this) the position of the objects is correctly indicated and they can be bisected with the certainty that the only circumstances which might vitiate the result are personal errors in estimating the bisections or errors of the micrometer itself.

The result, as we have said, is in terms of the inch, but if later on it be desired to convert the same into proportional parts of a millimetre, then the figures should be *divided* by ·03937, or *multiplied* by 25·4. If desired in microns [1] (μ), further *multiply* the result by 1000, or *divide* the original figures in inches by ·00003937, or *multiply* by 25,400.

If it be required in the first instance to obtain the measure in terms of the millimetre, the micrometer should be oriented by means of a stage micrometer divided in millimetres. Figures so obtained can be changed into microns by *multiplying* by a 1000.

To change millimetre readings into inches *multiply* by ·03937, and to convert microns into inches, by ·00003937.

If the object be a large one, and yet requires for certain reasons a high power, it will be found more convenient to set back the fiducial line some given distance—that is to say a certain number of notches in the comb. Mr. Nelson has arranged a micrometer for this special purpose.

What has been said explains the most accurate method of measurement: other methods are described in Chapter XVII., where the eikonometer and the measuring ocular are fully described.

Occasions may arise when the microscopist is desirous of obtaining the number of lines to the inch in a given specimen. Having obtained the value of one revolution of the screw, five lines (or more) are taken of the specimen—that is to say one

[1] The Micron, usually written μ, is the thousandth part of a millimetre: it will be explained further on.

wire is placed on number one line and the second on number six, and the distance carefully measured ; let us say they are contained in ·00006 of an inch. Then we say—

As ·00006 : 5 :: 1 = $\frac{5}{.00006}$ = 83,300 to the inch.

If desired in terms of a millimetre the figures must be divided by 25·4, thus showing that there are, roughly speaking, 3280 to the millimetre, and a little over 3 to the micron (which we have said is the thousandth part of the millimetre).

From what has been said it is obvious that the valuation of one turn of the micrometer must always be taken upon *every* use of the instrument—we mean of course if the microscope in the interval has been employed for other purposes in the ordinary way, and the micrometer eyepiece removed between different measurements being taken.

Units of Measurement

In the previous section the means of converting measures expressed in inches into terms of the millimetre have been given. The reason for so doing is the fact that a growing feeling is rapidly gaining acceptance for all dimensions in scientific matters to be expressed in the metrical system. Since microscopical objectives have in later years been so much improved, both in defining as well as separating power, the possibility of measuring small objects has become greater and greater. This being so, the inch as a unit of measure has been found to be inconveniently large seeing that some of the small objects, or portions of them to be measured, may be commensurate even with the wave-length of light. Consequently scientific authorities have sought about for a more suitable standard, one so much smaller than the inch that it would permit an expression of measurement of an object in less figures, and so in a more convenient form than hitherto. Selection fell on what is called the Micron—usually expressed by the Greek letter μ (*mu*)—which is the thousandth part of a millimetre, or the twenty-five thousand four hundredth part of an inch.[1]

[1] It should be mentioned here perhaps, although hardly in logical sequence, that even the μ or micron is not small enough a unit for the physicist when dealing with the measurements of the wave-lengths of light. In this case the German savants employ what is called the double *mu* (written $\mu\mu$), which

Seeing that there may be some readers to whom the metrical system may not immediately appeal, we append the following explanation in the hope it may furnish the information desired.

A metre was originally intended to be the $\frac{1}{10000000}$ part of the distance from the pole of the earth to the equator, measured along a given meridian. Owing however to an error, it is known now to be in reality too short; hence the metre, strictly speaking, is merely the length of a given definite standard kept in Paris.

In Fig. 154 the numeral 1—to represent the metre—is seen

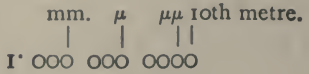


Fig. 154.

to be separated by the decimal point from the ten following cyphers. The third cypher indicates the position of the millimetre (written mm.) because it is the thousandth part of the metre. Hence this quantity is expressed in decimals (in terms of the metre) as 0·001. If we proceed to the sixth cypher we arrive at the micron (written μ), which is therefore the thousand-thousandth (millionth) part of the metre, whilst being the thousandth part of the millimetre. In decimals then it is expressed as ·000001 in terms of the metre, but as ·001 in those of the millimetre.

Continuing to the ninth cypher, we have the position of what is called the double *mu*—a still smaller unit of measurement—(written $\mu\mu$), which in decimal parts of a metre becomes 0·000000001, in that of a millimetre as 0·000001, and of a micron as 0·001.

The last cypher of all in the figure indicates the position of the smallest unit in existence, being the tenth part of the double *mu*. It is called the tenth-metre. In decimals, with respect to a metre, this is written 0·0000000001, in terms of a millimetre as 0·0000001, of a micron as 0·0001, and of a double *mu* as 0·1. Hence ten of these tenth-metres make one double *mu*, ten thousand a micron, and ten million a millimetre.

is the *thousandth part of the micron;* but the English scientist adopts a smaller unit still, called the tenth-metre, which is the *ten thousandth part of the micron*, the *raison d'être* of the term being that 10^{10} (10 at the tenth power) go to a metre.

As we have said already, the micron is the now almost universally adopted unit of measurement for the microscopist, but is found too large for spectroscopy when dealing with the wave-lengths of differently coloured light. We have mentioned too that in Germany the unit for this branch of science is the double *mu*, whilst in England it is the tenth-metre. The position of both these units has been shown with respect to the metre, but we might, to make our meaning quite clear, illustrate for a moment the difference of expression according to the two standards. Take the wave-length of the F line in the spectrum. In this country it would be written approximately as 4862,[1] measuring four thousand eight hundred and sixty-two tenth-metres, whilst in Germany it would appear as 486·2 double *mu*, or four hundred and eighty-six decimal two double *mu*. This will be readily understood by the reader remembering the change in the position of the decimal point between the two standards.

Although what follows has already been stated elsewhere in this book, still it may be convenient to repeat that at times it is a convenience to be at once able to convert tenth-metres into terms of the inch or *vice versâ*. This can readily be done by dividing 254,000,000[2] by the expression in tenth-metres. Thus 4862, the approximate length of the waves of F light in tenth-metres, would become—expressed in terms of the inch—as 52,241 waves to the inch—*i.e.* fifty-two thousand two hundred and forty-one; *vice versâ*, if it be desired to express in terms of tenth-metres a measurement written in those of the inch, the same nine-figured quantity must be divided by the quantity expressed in terms of the inch, when the quotient furnishes the measurement in tenth-metres. When dealing with expressions written in terms of double *mu*, one cypher must be taken off the above-mentioned nine-figured quantity, the proceeding for conversion—either way—being carried out in a similar manner.

Notwithstanding the explanation given for conversion of the inch and parts into the millimetre or micron or *vice versâ*, it may be sometimes convenient to use the following tables, even

[1] Decimal portions of the tenth-metre are added when great exactitude is required; hence the exact wave-length of a Fraunhoffer line in the F region might appear 4862·23.

[2] How these figures are obtained is shown in the Addenda.

if only as a check upon any conversion that might otherwise be made:

Table for Conversion of British and Metric Measures

μ.	inch
1 =	·000039
2 =	·000079
3 =	·000118
4 =	·000157
5 =	·000197
6 =	·000236
7 =	·000276
8 =	·000315
9 =	·000354
10 =	·000394
20 =	·000787
30 =	·001181
40 =	·001575
50 =	·001969
60 =	·002362
80 =	·003150
100 =	·003937
1000 =	1 mm.

mm.	inch
1	= ·039370
2	= ·078741
5	= ·196852
10 (1 cm.)	= ·393704
20	= ·787409
50	= 1·968522
100	= 1 decimetre

Example.—What is the equivalent in inches to 21 μ.?

20 μ. = ·000787
1 μ. = ·000039
·000826

a tenth-metre = a 250 millionth of an inch nearly.
a double mu = a 25 millionth „ „
a micron = a 25 thousandth „ „

Inches into Microns and Millimetres

inch	μ.
$\frac{1}{25400}$ =	1·015991
$\frac{1}{20000}$ =	1·269989
$\frac{1}{15000}$ =	1·693318
$\frac{1}{10000}$ =	2·539977
$\frac{1}{9000}$ =	2·822197
$\frac{1}{5000}$ =	5·079954
$\frac{1}{1000}$ =	25·399772

$\frac{1}{36000}$ =	·028222
$\frac{1}{32000}$ =	·031750
$\frac{1}{20000}$ =	·050800
$\frac{1}{4000}$ =	·253998
$\frac{1}{10}$ =	2·539977
$\frac{1}{8}$ =	3·174972
$\frac{1}{4}$ =	5·079954
$\frac{3}{8}$ =	9·524915

a micron (usually written μ) = $\frac{1}{1000}$ millimetre = ·00003937 inch.
a millimetre = $\frac{1}{10}$ centimetre = $\frac{1}{1000}$ metre = ·03937 „
a centimetre = $\frac{1}{10}$ decimetre = $\frac{1}{100}$ metre = ·39370 „
a decimetre = = $\frac{1}{10}$ metre = 3·93704 „

Inches and Millimetres

5000	lines per inch	=	197	lines per mm.	
10000	„ „	=	394	„	„
30000	„ „	=	1,181	„	„
50000	„ „	=	1,968	„	„

25399·77	lines in an inch	=	1	line to the	μ.
50799	„ „	=	2	lines to the	μ.
101599	„ „	=	4	„	„
152399	„ „	=	6	„	„
203198	„ „	=	8	„	„
253998	„ „	=	10	„	„

$\frac{1}{5000}$th	of an inch	=	5·08	μ.
$\frac{1}{10000}$	„	=	2·54	„
$\frac{1}{20000}$	„	=	1·27	„
$\frac{1}{50000}$	„	=	·508	„
$\frac{1}{70000}$	„	=	·363	„
$\frac{1}{100000}$	„	=	·254	„

Square	$\frac{1}{8}$	inch	=	10·08045	square millimetres.
„	$\frac{1}{10}$	„	=	6·45148	„ „
„	$\frac{1}{12}$	„	=	4 48021	„ „
„	$\frac{1}{100}$	„	=	·06451	„ „

Square		μ.	=	·00155	square $\frac{1}{1000}$ inch.
„	10	μ.	=	·1500	„ „ „
„	100	μ.	=	15·5003	„ „ „

Multiples of the above may be found by multiplying the values given by the square of the multiplier. Thus, square $\frac{4}{10}$ inch $= \frac{1}{10} \times 4$; the square of $4 = 4 \times 4 = 16$, and $6{\cdot}45148 \times 16 = 103{\cdot}22368$ square millimetres ("Carpenter" abridged).

Note.—In a "Treatise on the Diatomaceæ," by the late Dr. Henry Van Heurck, it will be found that he used a hundredth part of a millimetre (0·01 mm.) as his *unit of measurement*: one c.d.m., as he called it, = ·0003937 English inches = 10 μ (microns).

CHAPTER XIII

THE MICROSCOPE AND OBJECTIVES SUITABLE FOR DIFFERENT PURPOSES

THE selection of a suitable stand and objectives depends upon the special purpose for which they are intended to be employed. The different classes of subject will therefore be treated seriatim.

Botany, and as an Instrument for the Textile Trade

For these purposes the simplest type of stand is all that

Fig. 155.—Himmler's Stand.

is required. A good coarse adjustment should be provided preferably of the rack form, but the sliding tube will do very well if the microscopist is "handy with his hands." The fine adjustment is not really needed seeing the objectives are all very

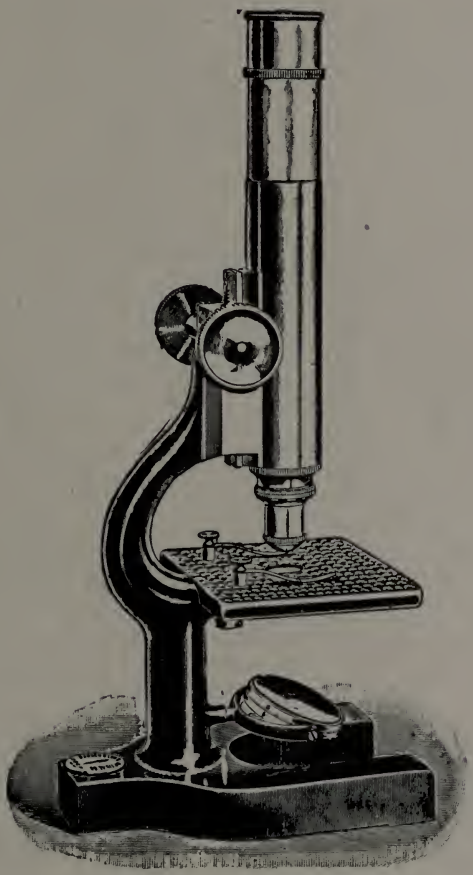


Fig. 156.—Bausch & Lomb's Stand A.

low powers, a 2-in., 1½-in. and 1-in., but it is not to be despised, especially when only a draw-tube takes the place of a rack form of coarse adjustment as above stated.

The mirror should have a concave and flat surface and also be provided with cap of opal glass that can take the place of

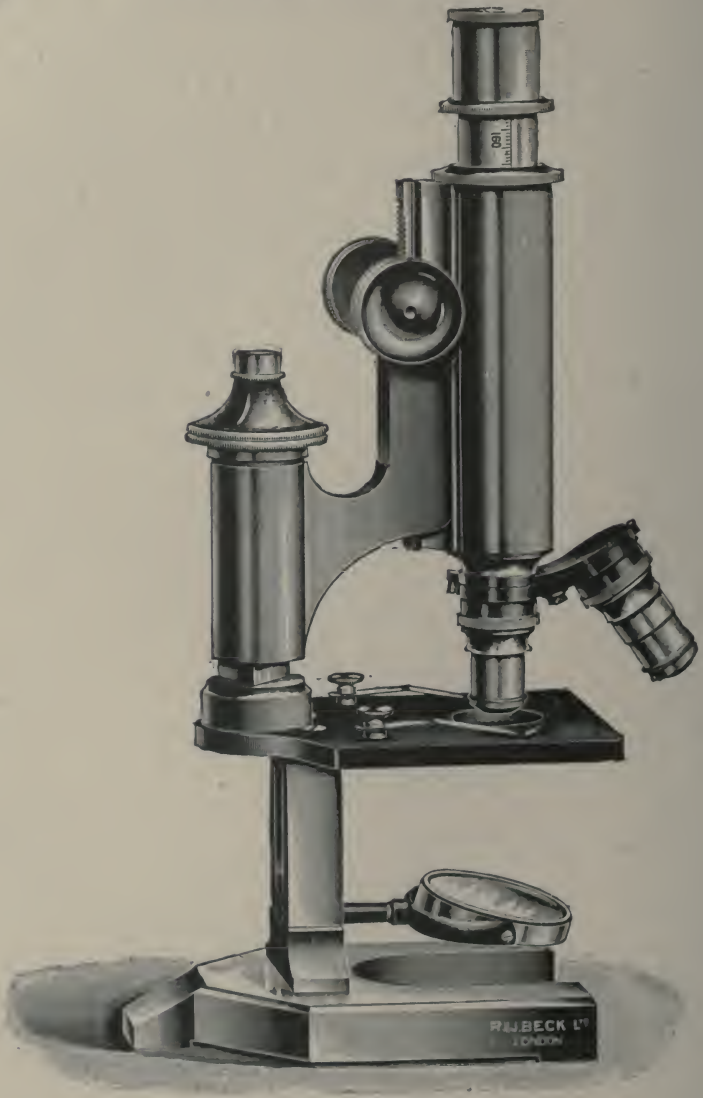


Fig. 157.—R. & J. Beck's No. 1124 Stand.

the silver surface when needed. This is usually employed[1] when the light is found to be too powerful and wants softening

[1] Occasionally it is used to obtain polarised light, as explained in the chapter devoted to that class of illumination.

down; but some botanists now prefer to use a No. 96 Kodak neutral tint film between the illuminant and mirror under these circumstances instead. The instrument need not have a joint, as an upright position is quite as convenient for botanical and textile

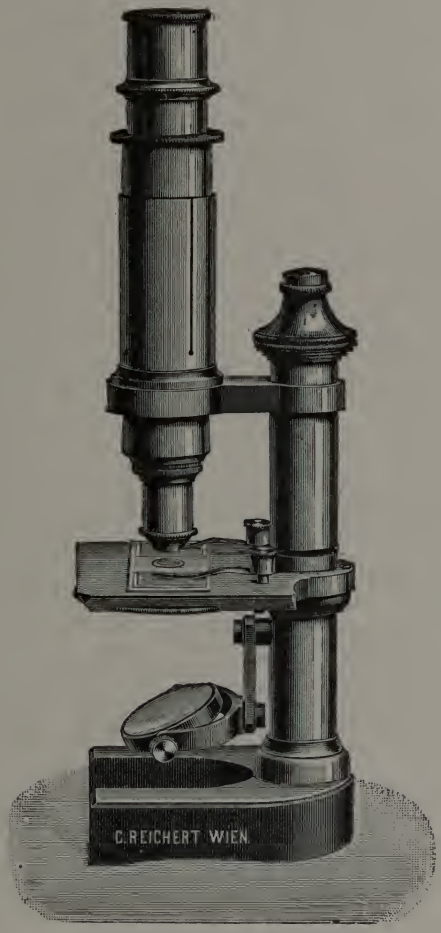


Fig. 158.—Reichert's Stand.

studies, indeed at times rather more so than an inclined one, for it prevents specimens, temporarily under examination, slipping off the stage. Otto Himmler (Fig. 155) used to make a cheap little stand, whilst one of much the same design is provided by Messrs. Bausch & Lomb, shown in Fig. 156, Stand A.

Messrs. R. & J. Beck supply a different and more perfect model (Fig. 157), called their No. 1124 Stand. It is a cheap form of their "London" Microscope, and has a fine adjustment in addition. Reichert (Fig. 158) used to sell a very firm type (non-inclinable) that has no rackwork to the coarse adjustment, but has a fine one, by which means the microscope can be used for slightly higher powers when required. It was an excellent little production, and reminds one of the original Oberhauser model.

Those who desire a modest form of stand, but one that is easy

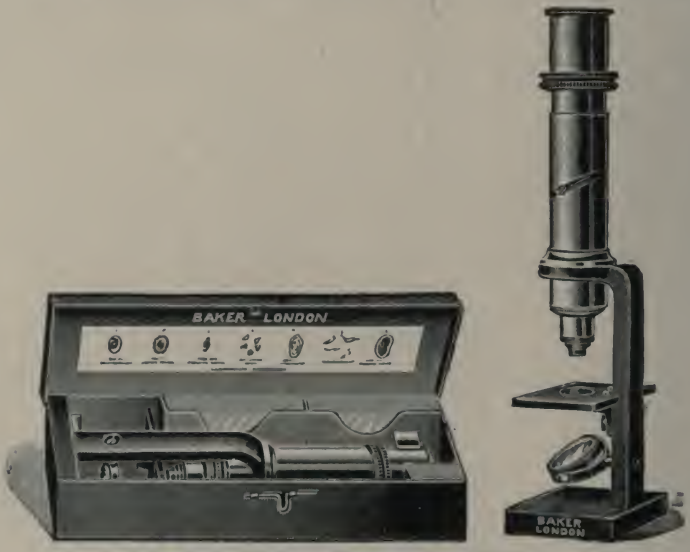


Fig. 159.—Baker's Plantation Microscope.

to carry about and suitable for *quite* low-power work, should see the Plantation Microscope made by C. Baker. It is not provided with a fine adjustment, and it has only a draw-tube for a coarse one. It drops into a case of exceedingly small dimensions (Fig. 159).

Most other manufacturers supply cheap stands that are equally efficacious.

The objectives required by the student in Botany and for the Textile Trade need not be of the very finest quality, and a 2-in., 1½-in., and a 1-in. are all that is necessary. Messrs. R. & J. Beck make a series of this description, and so do Messrs.

Watson & Sons, and others. If more perfect combinations are desired, most opticians' productions are equally excellent. The wide-angled Holoscopic objective 24 mm. N.A. 0·24 is a favourite lens, as it has such a very flat field, about $\frac{1}{10}$ in. diameter, whilst its great aperture $\left(\frac{F}{2}\right)$ makes it exceedingly serviceable for photography if used with a suitably deep green screen.[1] The Zeiss 35-mm. projection objective is, we have found, a useful combination, whilst the $1\frac{1}{2}$-in. of Beck and the 2-in of Wray[2] have given us great satisfaction for years. Zeiss used to make a useful combination that by simply turning a ring—rotating it like an objective collar—caused the magnifying power to be increased about double. This objective produced with ocular 1, a magnification of 3 to 8, whilst the limit is 33 when used with ocular 5, intermediate magnification being, of course, obtained by the use of the ring in conjunction with oculars of intervening powers. It is also sold by Koristka of Milan.

If the work the student is engaged upon demands the highest quality of definition, the apochromatic inch by Zeiss or that by Swift furnishes an excellent image when used with a compensating ocular. An illustration of the performance by the former is shown in Fig. 1, Plate XVI.

This arrangement forms a valuable one for photographing with, as no screen is required,[3] and such is the excellence of the objective that any compensating ocular can be employed to raise the magnification without producing any deterioration of the definition.

For Pharmacy, and as a Dairy Teacher's Microscope

For these purposes the student requires a rather more elaborate class of instrument, for he must expect to employ objectives up to a half-inch or even a quarter-inch. An Abbe (chromatic) type of condenser too is also required, but a modified form of non-

[1] The frontispiece was photographed with this objective and two green screens.

[2] This objective is, we believe, unobtainable now (except perhaps second-hand). It is regrettable, for, although it was originally designed for photography, still the under-correction necessary was scarcely noticeable owing to the focal length being so great.

[3] A piece of green-pot glass, or a 66, 56, or 61 green Kodak film often increases the contrast.

centring substage will in most cases fulfil all his requirements. Suitable stands are made by the following firms, although of course the list is not meant to be of an exhaustive nature. A particularly convenient variety is to be found in the model Histological Microscope by Baker, shown in Fig. 160; a superior

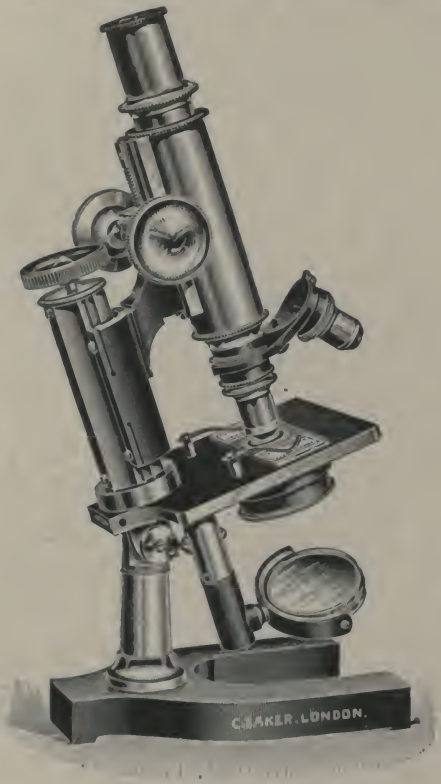


Fig. 160.

Fig. 161.

Baker's Histological Microscope.

one still is also made by the same firm. They can be supplied with a swinging substage, shown in Fig. 161.

In years gone by Leitz used to make a good stand, IIb; it is shown in Fig. 162. This microscope was an exceedingly useful piece of apparatus, and quite equal to the preceding.

A somewhat more elaborate and very solid stand is the "Fram," sold by Messrs. Watson & Sons, and it can be supplied

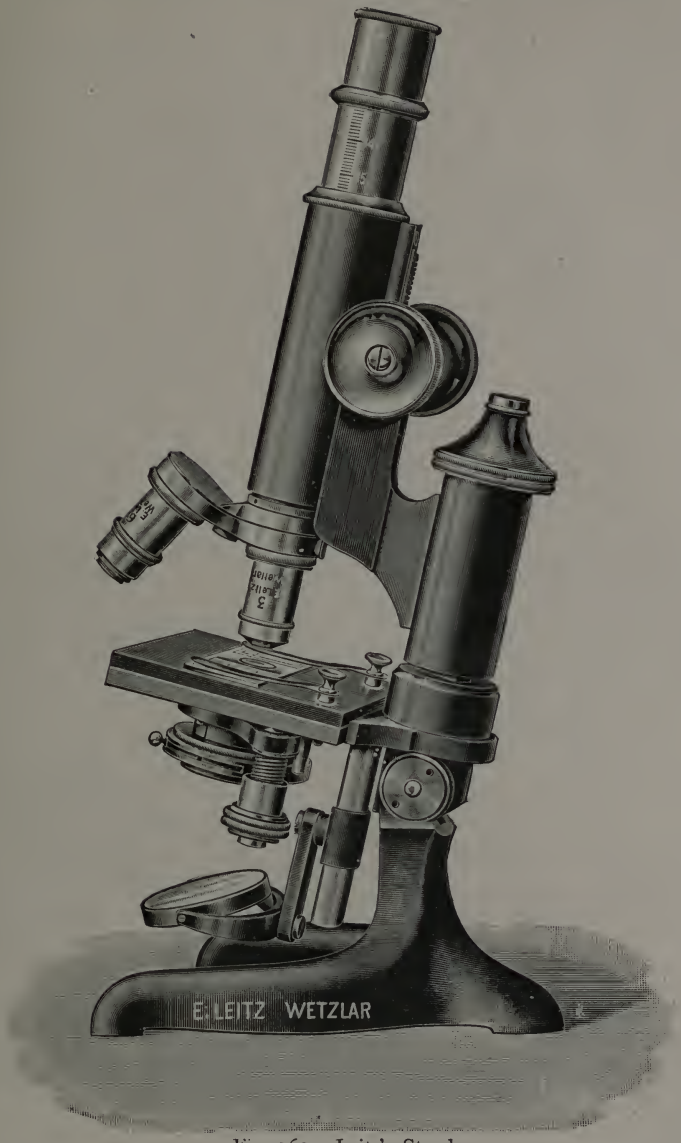


Fig. 162.—Leitz's Stand.

with substage arrangements of suitable quality when required. The illustration (Fig. 163) shows the excellency of the details and the general firmness of the model. Zeiss's Stand V.A is an

Fig. 163.—Watson's "Fram."

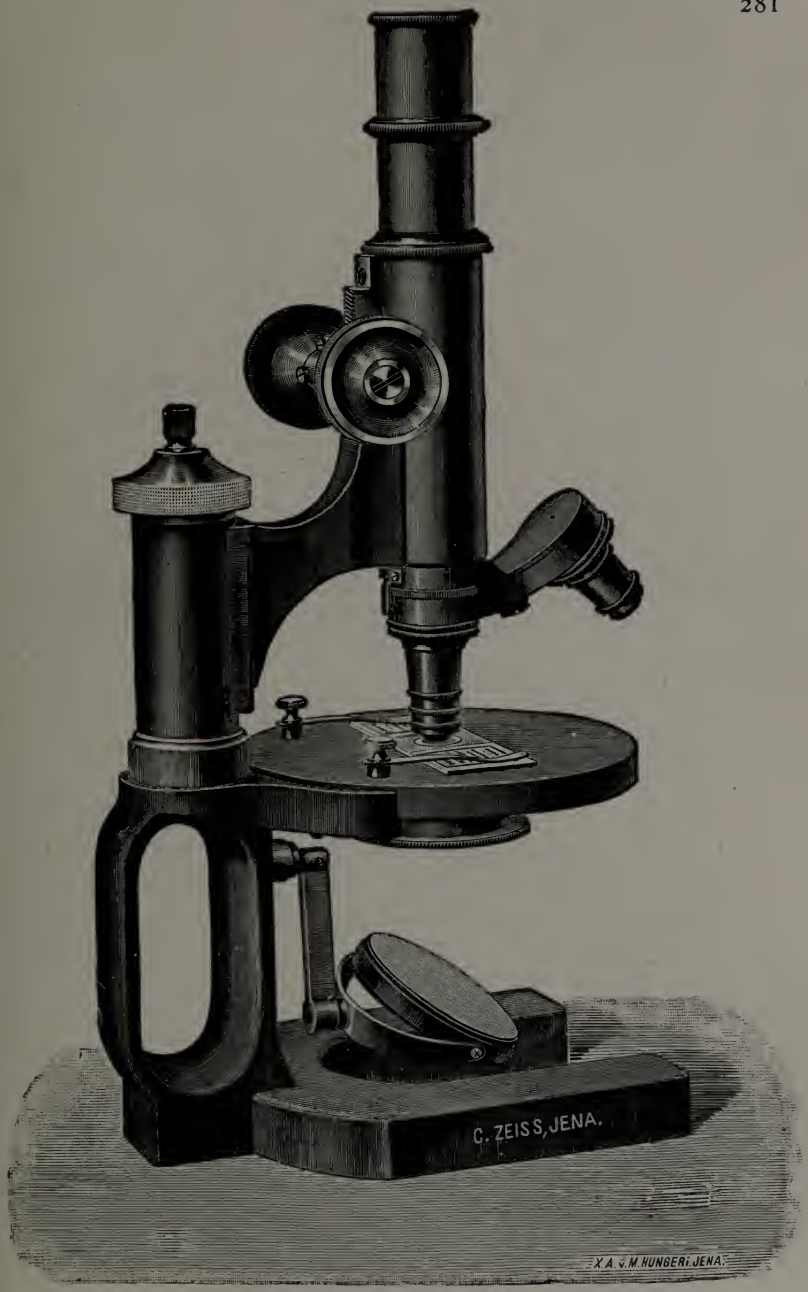


Fig. 164.—Zeiss's Stand V.A.

exceedingly useful type (Fig. 164). Constructed upon the latest (1905) lines, it is most easily held in the hand for carrying about.

The objectives for the study of **Pharmaceutical specimens** should be really good ones, an inch, $\frac{2}{3}$, $\frac{1}{2}$, and a $\frac{1}{4}$ being often used; but for dairy work the last mentioned is rarely required. The inch and the two-thirds are mostly of good quality with all opticians, but the finest achromatic combination $\frac{1}{2}$-in. it has been our lot to examine is that of the Holoscopic series by Watson & Sons. We have treated this lens in an absolutely cruel way in our endeavour to break down the image, but we have failed. Indeed, if employed with a green screen,[1] it is hard to distinguish any difference between the image it produces and that furnished by the $\frac{1}{2}$-in. apochromatic by Zeiss, employed in the same manner, and *that is saying a good deal.* A fine quarter is made by Swift, and we have much pleasure in saying that, with the same tests applied to it as to the Holoscopic we have just mentioned, we failed to break down the image, save perhaps under the most unfair conditions, which out of sheer desperation were tried. Reichert used to make an objective (a little over a quarter), called the *7a*, usually listed as a seventh, which was an exceptionally fine lens, especially for colour correction without loss of blackness in black-dot effects, and the extra magnification might at times be serviceable.[2] Leitz also used to make a first-rate combination of this focus, and all of Zeiss's low-power achromatics, which, to keep pace with modern nomenclature, should be called "semi-apochromats," were of the very finest excellence, and the images produced by them were *absolutely irreproachable in every respect.* Their sixth with a collar adjustment was a lens, if the pharmaceutical student desires more amplification than afforded by the quarter, well worth buying, as in our opinion it was one of the finest in the market, being only equalled by the Watson Holoscopic of the same focal length. We have tried to distinguish the performance of these lenses by every artifice possible, and have failed, and it is a matter for congratulation that competing opticians, as the outcome of their labours, should produce

[1] We have found that the pot green glass formerly recommended is more than equalled by the three Kodak green screens, 66, 56, and 61, used separately or conjointly. See Addenda.

[2] Those who will turn to the chapter upon the "Testing of Lenses" will better appreciate the meaning of this remark.

such magnificent results. The Zeiss has a little extra working-distance, which is an advantage sometimes, but the Watson is provided with a collar adjustment that admits of the objective being used as well on the short-tube instrument as on the long, which is a great convenience. Mention should also be made of a good sixth by Koristka, and another and very particularly fine semi-apochromat, made in pre-war times by Himmler of Berlin (6A), was a combination deserving the highest praise.

If the student desires to photograph his specimens, we recommend the half-inch and the quarter-inch apochromats by Zeiss, but it should be recollected both are only made for the long tube, so a lengthening adapter must be employed with the Continental form of instrument; or the transformer recommended by Dr. van Heurck (see Index). If he elect to employ only apochromats for the short tube, the third and sixth are extremely useful. Of these combinations the third by Koristka and Zeiss are most excellent lenses; and, with reference to the sixth, those by Reichert, Koristka, and Zeiss perform in a manner indistinguishable (see Plates at the end of the book).

The Brewer's Microscope

The Brewer needs a stand that will lend itself to the use of a twelfth, as his studies are carried into the subject of bacteria. We do not think, however, he need purchase such a fine instrument as that demanded by the bacteriologist (to be dealt with shortly), because the latter uses his instrument so much more than the former. Still, however, the Brewer's Microscope must be a good one; such, for example, as the very best employed for pharmaceutical purposes. We recommend the following: The small model No. 1129 of R. & J. Beck and Bausch & Lomb's BB stand, Baker's special Brewer's model, and one by Watson, all of which are arranged almost exclusively for the purpose.

With respect to objectives, those required by the pharmaceutical student suit the brewer, but the student will have to add a twelfth. We have already spoken of the first-mentioned combinations; but details concerning the semi-apochromatic twelfth will be found in the article devoted to medical purposes (Bacteriological section), and those relating to the

apochromatic 2-mm. in that devoted to critical work. We might mention that for some reason, not easily discoverable, a dry eighth or even a ninth seem favourite dry lenses for the Brewer.

For Medical Purposes

The student in Medicine has to use the instrument for Biology, Histology, Pathology, and Bacteriology. For the first two purposes nothing but a reasonably good stand is required, one indeed not quite so good as that we have recommended for the Pharmaceutical student of advanced type; but seeing that for Pathology and Bacteriology—especially the latter subject—a really fine, steady, and solid instrument is a *sine quâ non*, it is better for the beginner to purchase, once and for all, the best type of instrument *at the first*, rather than sell his cheap instrument at a loss when he requires a better one later on in his career, for the purposes we are now about to consider.

The Pathological and Bacteriological stands—for they may be considered together—should have a very large stage, one in fact far larger than needed by the ordinary user of the microscope. Seeing it has to accommodate what may be called the mostly used size of Petrie dish, its dimensions should never be less than six inches square, and larger would be of greater convenience still. It is a subject of regret that manufacturers do not grasp this necessity, and we very earnestly call their attention to it, for in some of the stands we are recommending now the stages are far too small.[1] We are fully aware arranging for so large a stage to a microscope involves a good deal of trouble, as it demands: a *longer* arm to hold the tube further from the body; a consequent alteration in the mechanical details of the fine adjustment; and a reconstruction of the understage arrangements. When this is grasped it becomes readily understood why the addition we speak of has not been hastily undertaken by opticians, for it adds to the cost of the instrument and involves considerable thought and trouble to arrange. We particularly call attention to this matter, because it has come to our knowledge that some students, those who happen not

[1] Since the first edition of this work was published, considerable advance has been made in this direction, many opticians having in recent models greatly increased the size of the stage.

to be possessed of mechanical knowledge, having asked the manufacturers "*just* to alter their stage to take a Petrie dish," have been somewhat surprised and annoyed at being told that

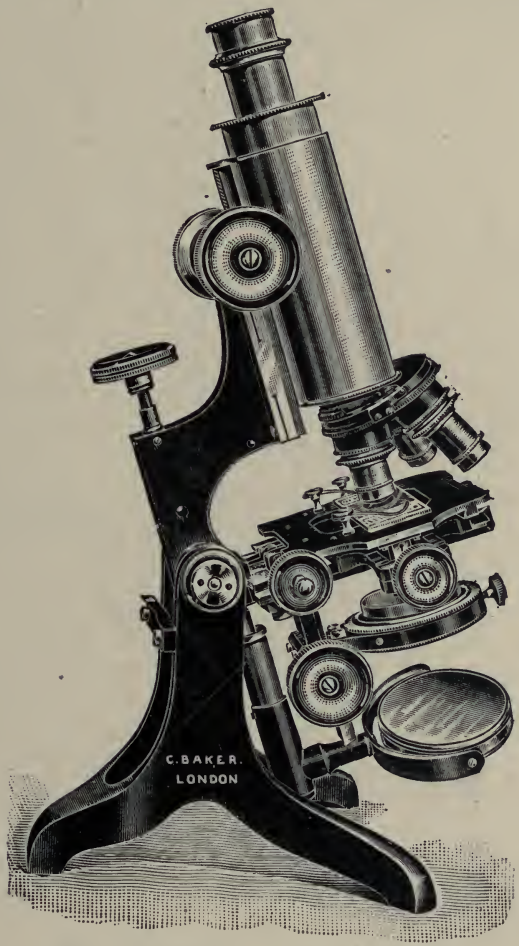


Fig. 165.—Baker's "D.P.H." Microscope.

such could not possibly be done with their instruments in question The difficulty is obvious when what we have stated is understood.

The stands which we believe are suitable for the purpose of the Bacteriologist are several in number—arranged in alphabetical order. We commence with that by C. Baker (Fig. 165),

called the "D.P.H." Microscope, which is a thoroughly sound and useful model, and one that has given great satisfaction; whilst Messrs. Bausch & Lomb, Rochester, N.Y., U.S.A. (English agents: Messrs. A. E. Staley & Co., Thavies Inn, E.C.), make

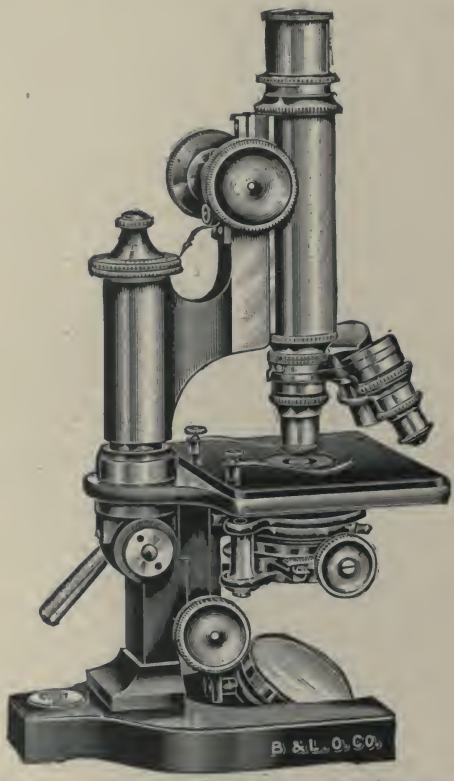


Fig. 166.—Bausch & Lomb's "C.A." Microscope.

another, called "C.A.," with exceptionally large stage (Fig. 166), which is also well spoken of.

Messrs. R. & J. Beck have quite recently introduced a new and improved model of their "London" microscope, called the "Regent Model." It has a stage 4 × 4 in. (surfaced with ebonite) which is provided with a special iris diaphragm of use with unstained specimens. Additional space is allowed between the stage and the base, which is certainly a great convenience (Fig. 167).

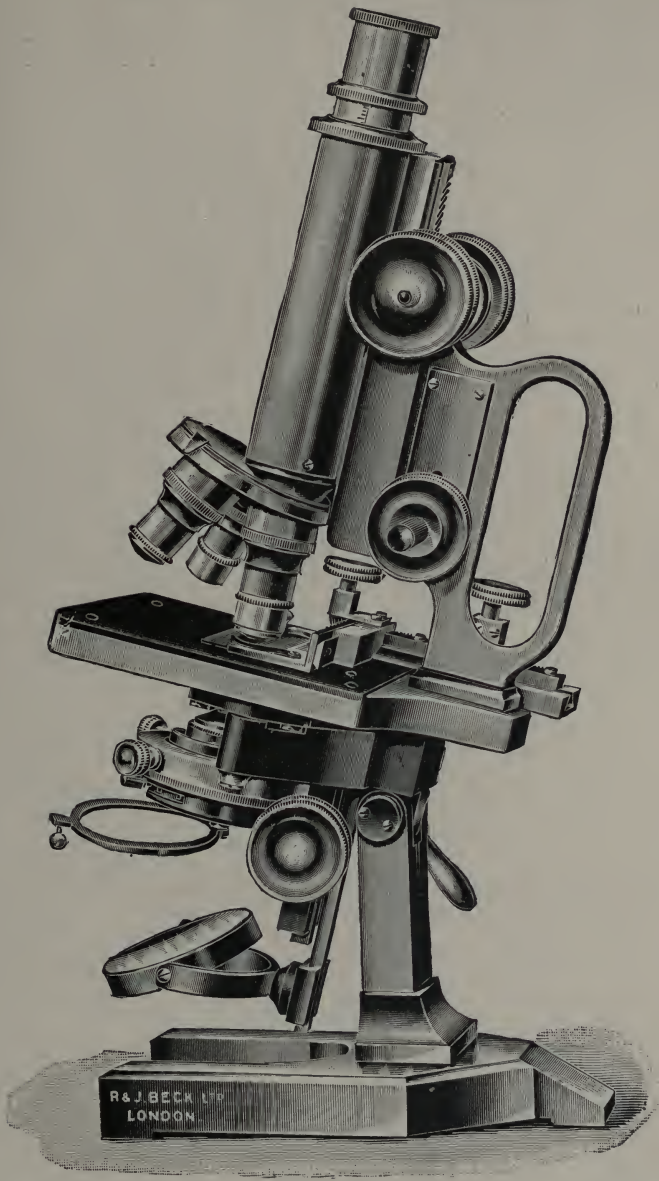


Fig. 167.—R. & J. Beck's "Regent Model" of the "London" Microscope.

Signor Koristka, an Italian maker resident in Milan, not much known in this country, but whose work is of the most

excellent type, we believe supplies a stand especially constructed for numerous bacteriological laboratories abroad, having a

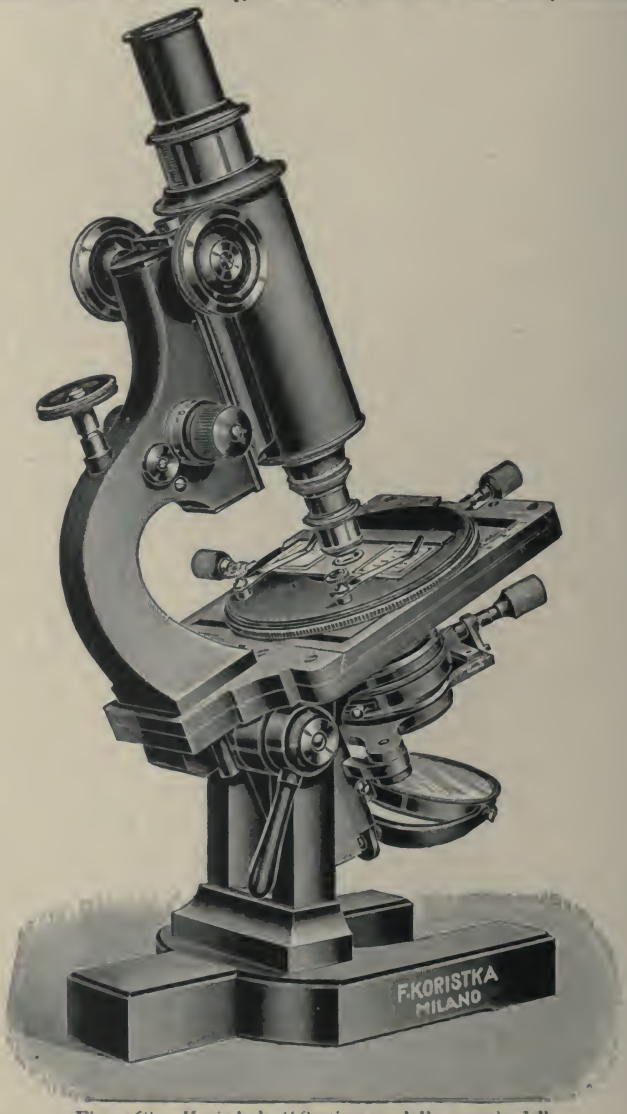


Fig. 168.—Koristka's "Stativo modello grande I."

vulcanite stage of large dimensions (Fig. 168). It is called "Stativo modello grande I."

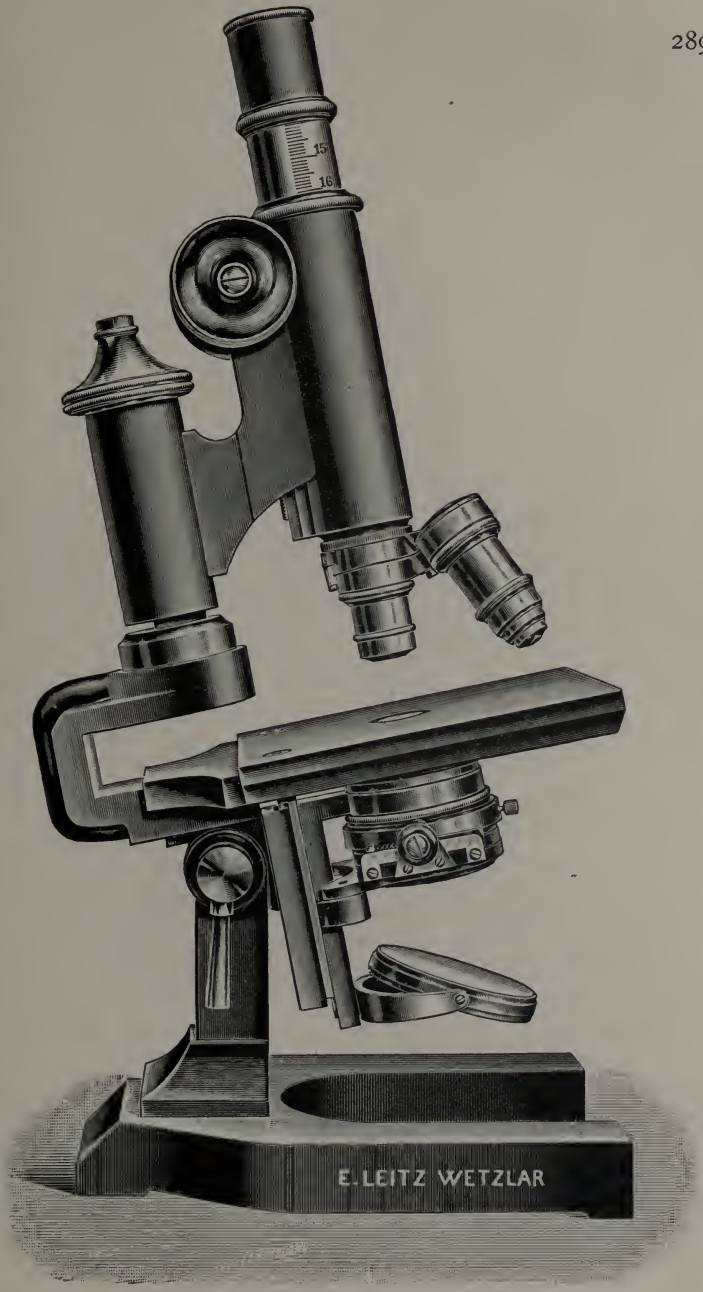


Fig. 169. —" Dölken's Microscope."

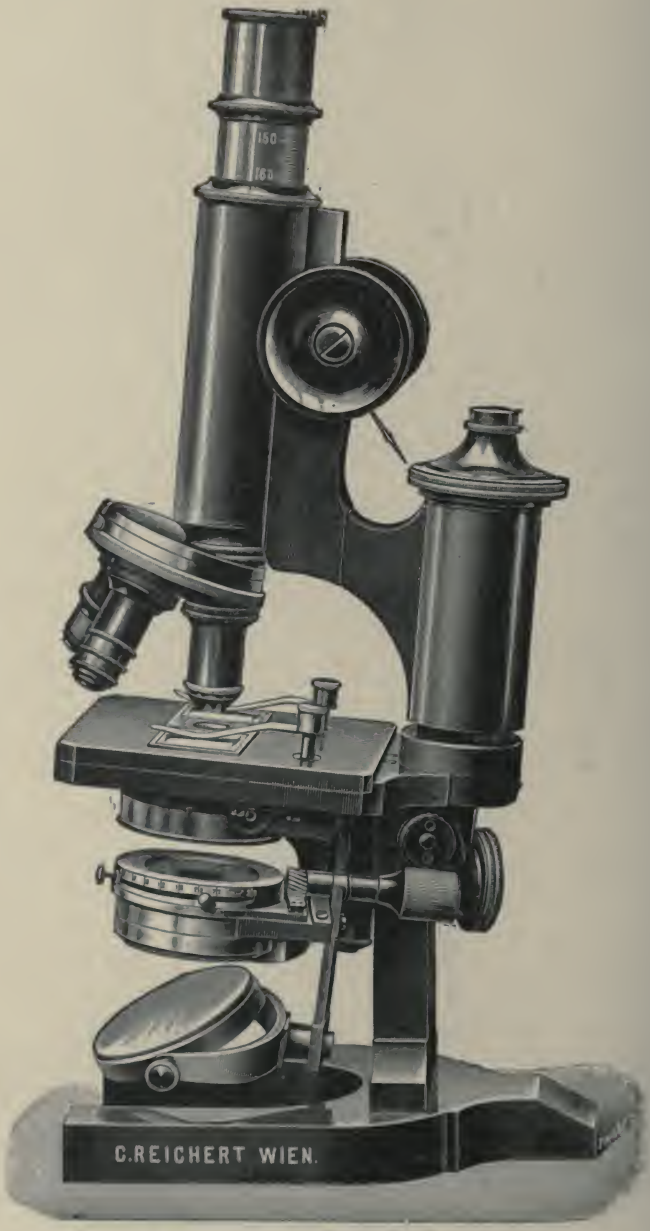


Fig. 170.—Reichert's "IIb Model."

The well-known firm of Leitz used to supply a special model which had a stage of considerable size peculiarly constructed so as to admit of exceedingly large preparations like brain sections. It is called "Dölken's Microscope" (Fig. 169). We believe this stand has given very great satisfaction, especially for the particular purposes for which it was designed. This model was further improved before the war.

Reichert furnished an instrument called his "IIB Model," with a vulcanite stage 90 by 95 mm. (Fig. 170). It had a swing-out substage condenser, and was spoken very highly of.

The Spencer Lens Co., of Buffalo, N.Y., also sell a very fine stand which is much sought after in America ; it is named "No. 40," and shown in Fig. 171. It has a large stage.

Messrs. Swift & Sons construct a special bacteriological stand of considerable excellence, which was selected for the laboratory of the ship *Discovery*, of Antarctic fame, from which it takes its name. It is shown in Fig. 172. The substage arrangement as represented is exceedingly primitive, but there is no doubt it could be added to.

Messrs. Watson & Sons' "Edinburgh Students' Model," a well-known instrument, still enjoys a great fame, and their recently designed "Bactil" does not seem to have taken its place in the manner some anticipated. The size of the top plate of the stage is approximately $3\frac{3}{8}$ in. square and is covered with vulcanite. Everything is introduced to produce great steadiness and rigidity (Fig. 173).

Carl Zeiss's "Model III.," although of the old type, was a most excellent one, being a practical and useful stand. We know of no fault with this arrangement save that it is so unpleasant to take hold of. This is entirely remedied in their 1905 model, which, besides being of an entirely novel construction as to form, contains the firm's new fine adjustment, which is perhaps one of the best in existence (Fig. 174).

It seems a matter of considerable difference of opinion amongst bacteriologists as to whether a mechanical stage is wanted for everyday work or not. Some think that it is objectionable because, if, when using corrosive chemicals—such as hydrochloric or acetic acids—any of the fluid runs into the slides of the stage, or upon the finely made screws, such are seriously injured unless immediately cleaned, which is a nuisance

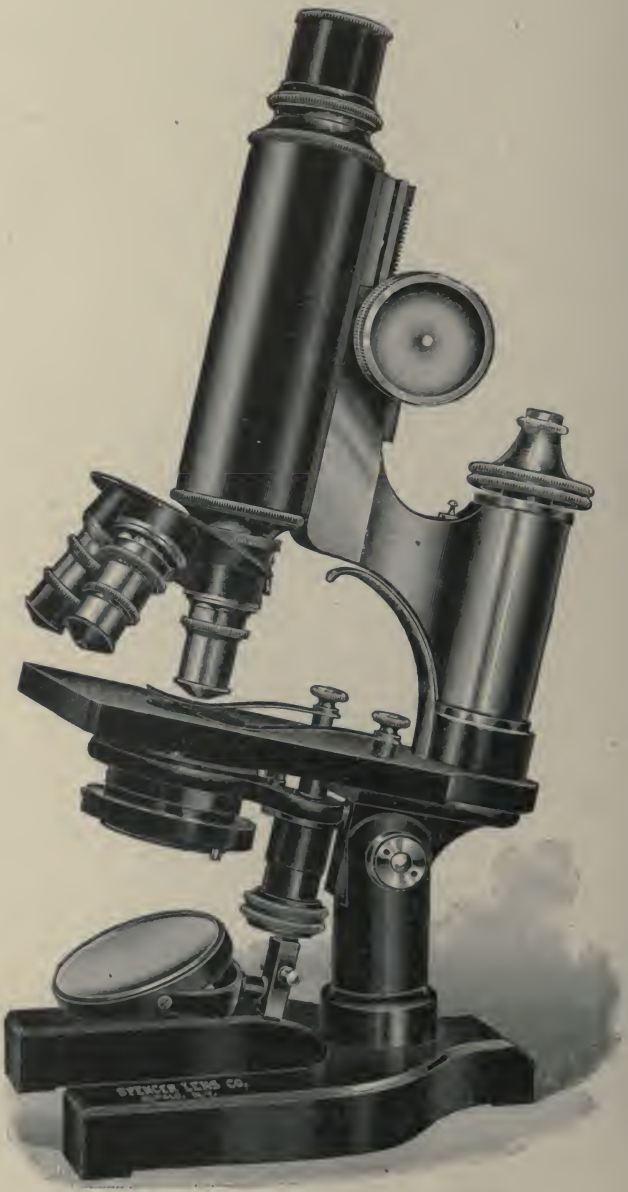


Fig 171.—The Spencer Lens Co.'s "No. 40."

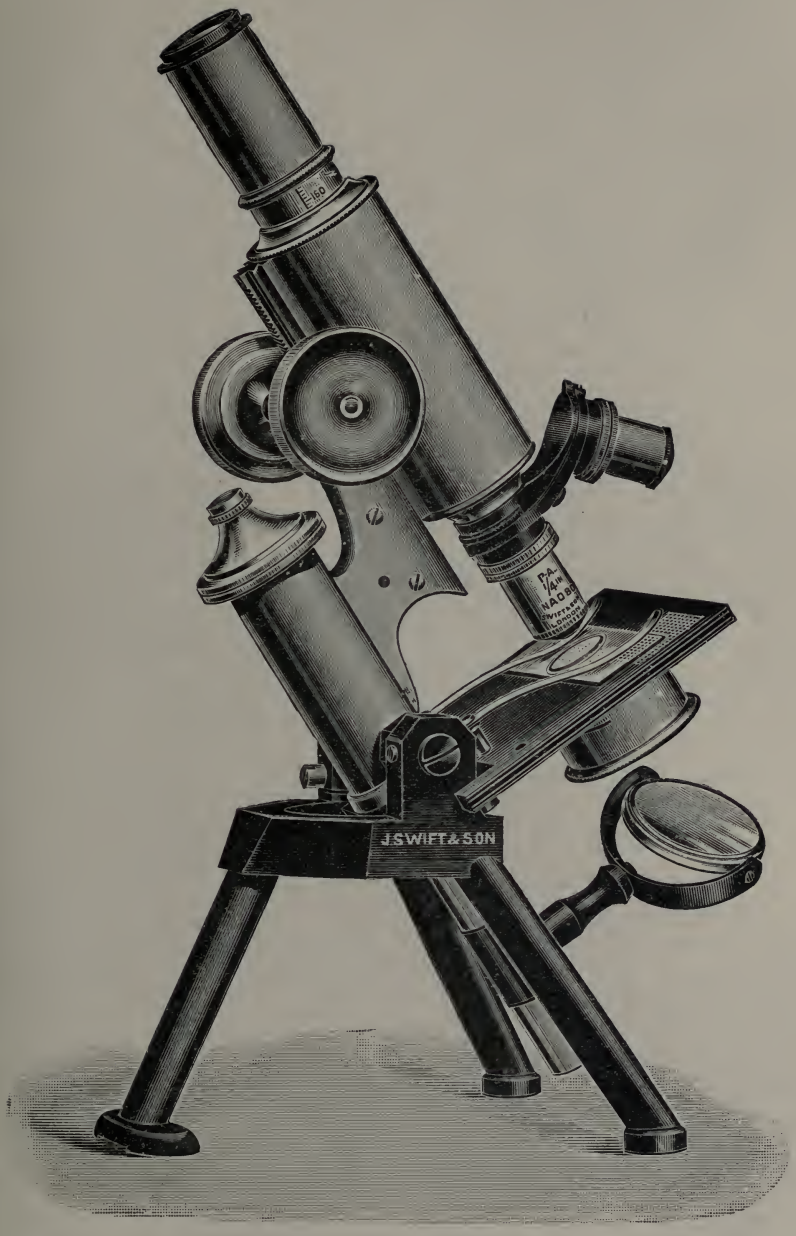


Fig. 172.—Swift & Son's "Discovery" Microscope.

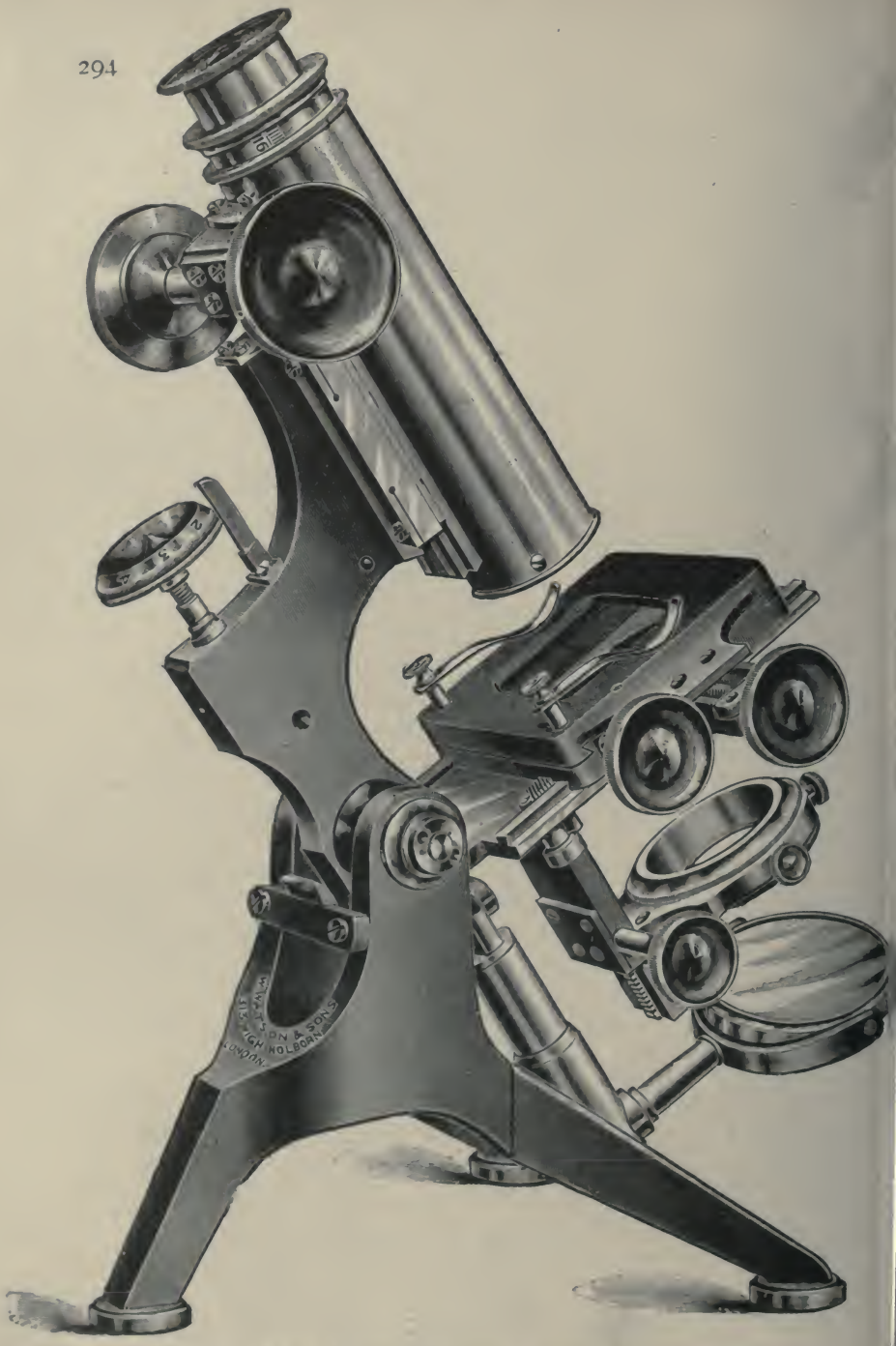


Fig. 173.—Watson & Sons' "Edinburgh Students' Model."

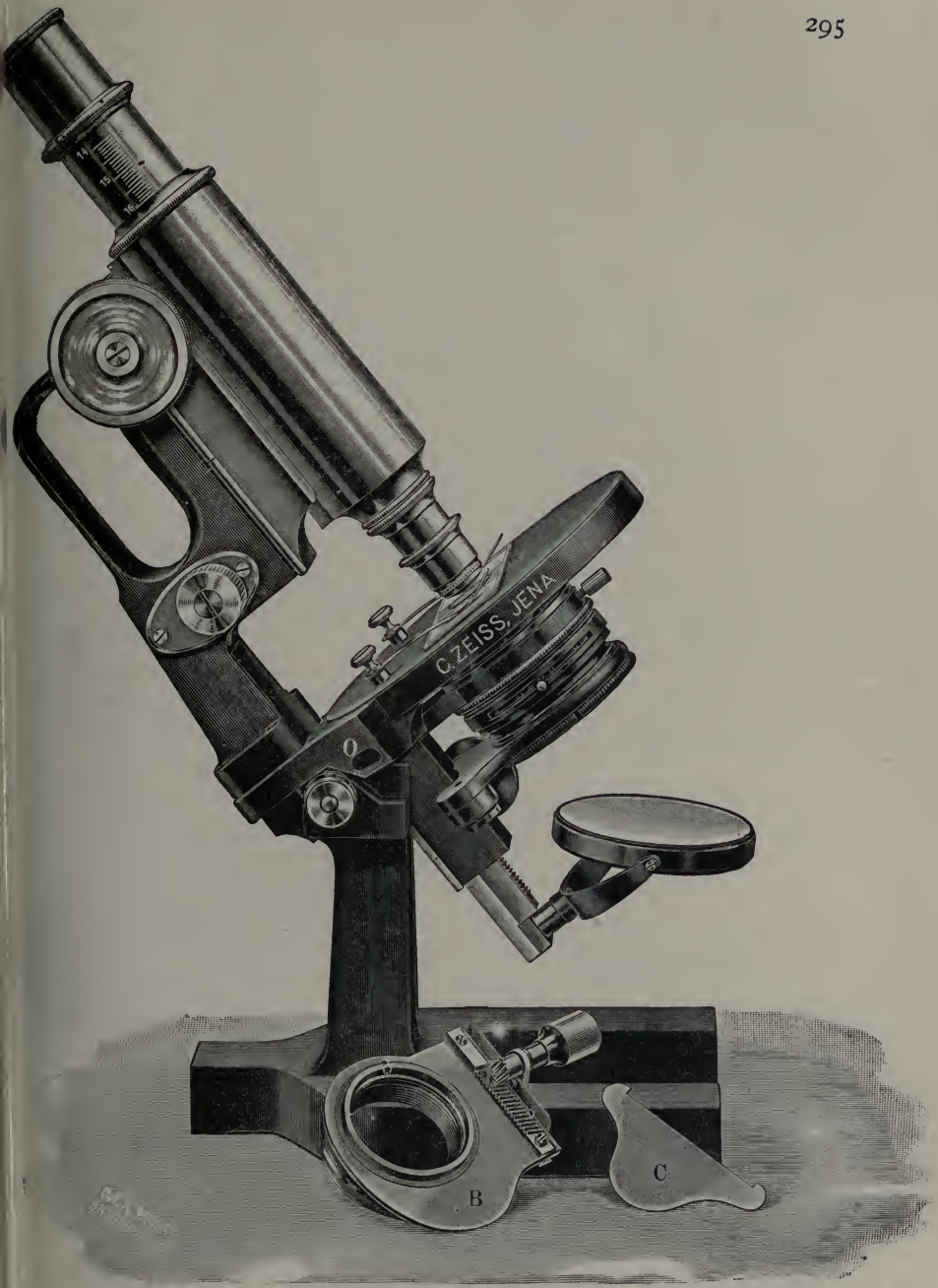


Fig. 174.—Carl Zeiss's "1905 Model."

and a hindrance. Others are of exactly opposite opinion, notwithstanding the objections just mentioned, holding that with reasonable care such accidents should never happen. A third class, however, seem to take an intermediate position, considering that for everyday work nothing mechanical is required, but that facilities should be always at hand to place upon the stage one of the numerous forms of auxiliary mechanical contrivances already described, so as to obtain the advantages derivable therefrom when absolutely necessary; as, for example, in making a "blood count," or when it may be required to hunt *seriatim* through a specimen.

In recommending an auxiliary stage, however, the remarks made when speaking of the different kinds in a previous part of this work should not be overlooked. We recommend them for the special object just mentioned, their performance for these occasional purposes leaving little to be desired; but when they are proposed for use *regularly* in the place of the stage built into the microscope—when they are, in fact, to be *daily* used, and *more especially to register by their verniers important positions in particular specimens*—we abruptly discontinue our recommendation. After a little use, the continued taking off and putting on to the stage appreciably alter their register with the axis of the tube, and hence positions obtained to-day may very likely be of no use a month hence. Besides, their mechanical construction is not such as to recommend them for hard work, a rapid "loss of way" and an increasing "shake" becoming more and more manifest when they are submitted to the heavy work of a laboratory. (For Auxiliary Stages, see pages 37-41.)

As regards the fine adjustment of the bacteriological microscope, we think it is pretty universally acknowledged one of medium speed is the best, unless the instrument is intended for photomicrographical use, when the finest is usually desirable.

The objectives mostly required by the student are the inch (or two-thirds), a sixth, and a semi-apochromatic twelfth; a 2-mm. apochromatic being added where expense is no object. We have no more to say about the selection of an inch, two-thirds, and a sixth than mentioned in the preceding sections; but we ought to remind the student it is very useful—indeed in some instances it is actually essential—to have an additional

sixth, one that has an *extra* long working-distance. It is for this reason. When employing a hæmocytometer to make a blood-count, it not infrequently happens that the microscopist has the unpleasant experience of finding he cannot focus the blood corpuscles with the sixth he employs for other purposes, owing to the special cover-glass provided with the apparatus being so extremely thick. Accidents may and often do occur from this cause. Since the first edition of this book, two sixths of especially long working-distance have come before our notice, being designed almost entirely for the purpose in question. One is by the Spencer Lens Co., of Buffalo, N.Y., and the other by Messrs. Watson & Sons, of Holborn, London. Each of these combinations has a working-distance of a millimetre, which may be said, roughly speaking, is double that which ordinarily obtains ; hence with either objective no accident or inconvenience should possibly occur. The numerical aperture of both these remarkably cheap and useful lenses is small, it is true—although quite sufficient for the purpose in question—but we mention it because the makers do not intend these objectives to compete in powers of resolution with others sold at higher prices and with greater aperture. Reichert made in pre-war times a "double focal-length sixth for counting blood corpuscles" called "No. 6B." This is a very excellent combination of decidedly superior resolving power to either of the two previously mentioned, but to counterbalance this its actual working-distance is, we find by direct measurement, about ·6 to ·7 mm., which we cannot help thinking is rather too small for the special purpose in hand. It is only fair, however, to add that Herr Reichert differs from us in this opinion, considering it meets the requirements of all cases quite effectively.

Lastly, it is necessary to add, owing to the recent extension of dark-ground illumination (by the construction and use of the new *reflecting* substage condenser of high numerical equivalent) to the use of high powers, that those who wish to investigate and study bacteria in their recent and living state, must add an apochromatic sixth to their battery of lenses, and perhaps a 2-mm. of the same type (with its limiting diaphragm especially constructed for the purpose), as the semi-apochromat produces an image so unpleasantly coloured by the secondary spectrum as to render it very objectionable to use.

As regards the twelfth, it may be considered as true that for the *general* use of the laboratory the microscope is mostly employed to distinguish differences of *form* rather than refinements of *detail*, so that really a very highly apertured twelfth is practically not needed. This is readily understood to be the case from the simple fact that nothing but dry condensers are ever seen in the bacteriological laboratory under its normal working conditions. This implies, of course, that no matter what the aperture of the objective be that is in use, whether a 1·25, 1·30, 1·35, or 1·40, it only works with a dry condenser *nominally* at N.A. 1·0.[1] We fear that this fact may have escaped the attention of some users of the microscope, who, in a passing moment of laxity of thought, have dwelt upon the advisability of having immersion objectives of N.A. 1·33 because of their high numerical aperture! Seeing that combinations working at N.A. 1·2 are so much cheaper than those ranging from N.A. 1·30 to 1·40, and that accidents to the former cost so much less to rectify than with the latter, we cannot help thinking this cheaper type of lens, *provided the quality is as good*, is all that is necessary for the rough hard work of the *student* in bacteriology. When, however, structural formations are under the consideration of professors, and when the highest resolution and very likely the highest magnification possible are sought after for the elucidation of certain details, *then*, instead of using a 1·35 or 1·40 semi-apochromatic, we venture to point out the real utility of the 2-mm. apochromatic with the oiled condenser steps in. We mean that it should be used as a court of appeal. In making these remarks we are fully aware our suggestions may be at variance with the ideas held by those doubtless better able to judge. Some, for example, whilst agreeing with what we have proposed in theory, think in actual practice it is more economical that the 1·35 semi-apochromatic should be purchased, and that it should be of the highest quality obtainable; and that, although the combination is cut down, it is true, by the dry condenser, to really work usually at *about* N.A. 1·0,[1] that it can be used at full aperture when so required by merely oiling the slip to the condenser, provided such illuminator be of the immersion type. To this we feel bound to demur. It may be advisable to

[1] The reader's attention is *particularly* directed to the chapter on the "Use and Abuse of the Substage Diaphragm" with respect to this matter.

buy the high aperture, because perhaps it performs better when cut down than an objective made to work at N.A. 1·10 or N.A. 1·20 would do, and to this we agree; but to think for one moment its full working aperture as *a court of appeal* is equal to an apochromatic where all the secondary spectrum is eliminated and the zonal corrections are brought to their highest correction possible, is to our mind an opinion too hastily arrived at. If both objectives are used with green film screens, the Gifford's F-line, or the pot-green glass recommended by us before, *then*, and *only then*, we do allow the performance of the two objectives may be difficult, save to the experienced eye, to disseverate. But can this always be done? It certainly cannot with many different kinds of staining, for the green screen will not suit every colour; neither is it at all times desirable to employ monochromatic light, especially when similar shaped objects can only be differentiated by their *selective* powers of absorbing different coloured stains, in which case light of one colour might be not only inconvenient, but possibly misleading.

Further, it has been suggested that water immersions, seeing they have a numerical aperture of 1·20, should suffice for ordinary bacteriological work. We must confess to have fallen in with that opinion at first, if only for the fact that *they need no wiping after use* (as distilled water dries without leaving any stain), *or the specimens either*, a very considerable inducement for their adoption. But one fatal objection, however, has become apparent to us in the practical application of the water objective before recommending its adoption, and it is this. Seeing the refractive index of the ordinary cover-glass is approximately 1·5, and that water is only 1·33, an alteration has to be effected in the length of the draw-tube (or the collar adjustment must be altered), *for every different thickness of cover* with which the objective is *employed*—that is, if the best performance be required. This is at once a nuisance and a hindrance in daily work, and one never required (save under very unusual circumstances) with the *homogeneous* immersion, simply because the cedar oil and the cover-glass having both approximately the same index of refraction, small differences in thickness of cover cause no appreciable effect in the definition of the object.

There is another objection to using a water immersion. It

is this. For many years it has been the habit in laboratories to avoid using cover glasses whenever possible, the front lens being oiled directly to the slip. It is found the oil does not in most instances injure the preparation, and definition is not affected because it has the same refractive index as the cover. If water be used—putting aside its possible solvent action on some preparations—we have before pointed out it certainly demands the adjustment of the draw-tube in length to compensate for the absence of the cover. Hence then it is obvious, all things considered, the water immersion is a mistake for bacteriological purposes.

In selecting a twelfth for bacteriology, a large working distance is a great convenience, for the constant use of the instrument—not, by the way, as a pastime, but as a daily routine—is very apt to breed a certain amount of contempt, if not a certain amount of carelessness, in the use of this delicate combination. Hence we do not unfrequently hear of broken covers, and, what is worse still, of crushed-in fronts, especially by commencing students, who may have never even seen a microscope before or used a high power.

Swift makes a twelfth with long-working distance which is a great favourite, and Watson their new "Versalic" with a working distance of ·43 mm., having its front fixed by some new design to prevent it being easily pushed in. Combinations by Koristka, Powell & Lealand, Bausch & Lomb, Baker, Beck, and by Watson (parachromatic series) are all good lenses.

All of these give good results, although varying somewhat in quality, but most of the same manufacturers make finer combinations at greater expense: such as Watson's Holos, which, with green illumination, furnishes a result that competes with the performance of an apochromatic.

Of the immersion twelfth with a *low* numerical aperture, such as N.A. 1·1 or thereabouts—which are often of use—we ought to mention two, those by Messrs. R. & J. Beck and by Messrs. Watson & Sons, which, seeing their price was in pre-war times so moderate, are really excellent constructions.

Some bacteriologists complain of feeling considerable fatigue in the eye when they have for long periods to examine specimens, as necessary, for example, when searching for the presence or absence of bacilli tuberculosis in several specimens. The comfort of using a suitable monochromatic screen is not so generally

known or fully appreciated as it should be. The best we know is either the green film screen by Kodak, Ltd., or the pot-green glass[1] of which we have already spoken. Either of these makes a red-stained organism quite black and very easily detected, the field being a soft monochromatic green ; whilst both these contrast-screens afford very great relief, and enable the observer to use his eye much longer and with much less fatigue. In the case of bacteria that are not of a red colour, however, these filters are not of quite so much use—for example, with specimens stained with methylene blue. Here a contrast-screen of great convenience is formed by using a gelatine plate stained with aurantia. An easy way of preparing such is to place an ordinary lantern side-plate straight out of its box into an aqueous solution of any strength of hyposulphite of soda (of course in the dark-room) until it completely clears, and after washing well in water, say for half an hour, into a solution (aqueous) of the dye mentioned, film uppermost. Left in this solution all night, it is *rinsed* the next morning in plain water and allowed to dry. Should a single glass not be of sufficiently deep a colour, two may be united by Canada balsam and dried. If the microscopist finds the heat of his lamp is apt to melt this screen it should be, after the rinsing, allowed to rest in a solution of formalin (equal parts of formalin and water) for about five minutes and then dried. This may make the colour much fainter. To meet the difficulty it is best to use a solution of the aurantia made with spirits of wine and water, which will stain the gelatine very much more than will the simple aqueous solution, and consequently the screen is of a deeper colour after treating with the formalin.

Too powerful a light is really not required by the bacteriologist. Some are of opinion it is the fault of the five-shilling Nernst lamp for ordinary work. A substitute, we have elsewhere stated, is to use the 32 candle-power Zig-zag lamp of Stearn's make (because of his peculiar arrangement of the filament) with an intervening slip of ground glass, or the globe of the lamp itself "sand" frosted. Some laboratories use the ordinary oil-lamp, whilst others prefer an incandescent gas-lamp to all other

[1] The green screens are called No. 66, 56, or 61. The special green glass, sold by Baker or Watson, of Holborn, London, must not be confused with what is called "Signal Green," which is different.

illuminants; but their great heat offers a considerable objection, in our opinion, to their use.

The remarks to those about to commence with the microscope concerning not only *keeping both eyes open*, but *properly focussing them both also*, are well worthy of perusal by the commencing student, and may even be of comfort to those more experienced, saving them from having diplopia or double vision, which occasionally makes itself manifest, and which has been thought by some to arise from this cause.

The Petrological Microscope

The particular application of the microscope to the subject of petrology has been growing of late; but until recent years the special requirements of the petrologist—which are not a few in number—have never been found embodied in one instrument alone. Several manufacturers have now, however, taken up the subject somewhat warmly; hence several highly complicated and beautifully ingenious stands have recently been put upon the market. We mention a few only of the more modern. In doing this we have thought it desirable to state in several instances more details than we have hitherto mentioned in the previous stands, where the figures mostly explain themselves.

The first is by Messrs. Bausch & Lomb, of Rochester, N.Y. (Fig. 175), English agents: 37-38, Hatton Garden, London, E.C.1, which we briefly describe as follows:—

Base, horseshoe form.

Pillar, finished same as base.

Stage, circular, revolving, with vulcanite stage-plate, having scales graduated in millimetres at right angles for the location of the specimen, spring clips, circumference graduated to 360°, with vernier; centring screws.

Substage, a modification of the complete substage, with rack and pinion adjustment for vertical movement.

Focussing adjustments, coarse adjustment by standard rack and pinion; fine adjustment by standard micrometer screw movement, having pointer and graduated milled head of extra large size.

Adjustment of Bertrand's lens by rack and pinion.

Main tube with universal thread, slots for quartz wedge and Bertrand's lens, sliding prism-box, iris diaphragm between prism-box and Bertrand's lens, draw-tube nickelled, carrying standard size eyepieces. Nosepiece with centring adjustment.

Polarising apparatus. The polariser and analyser are extra large Nicol

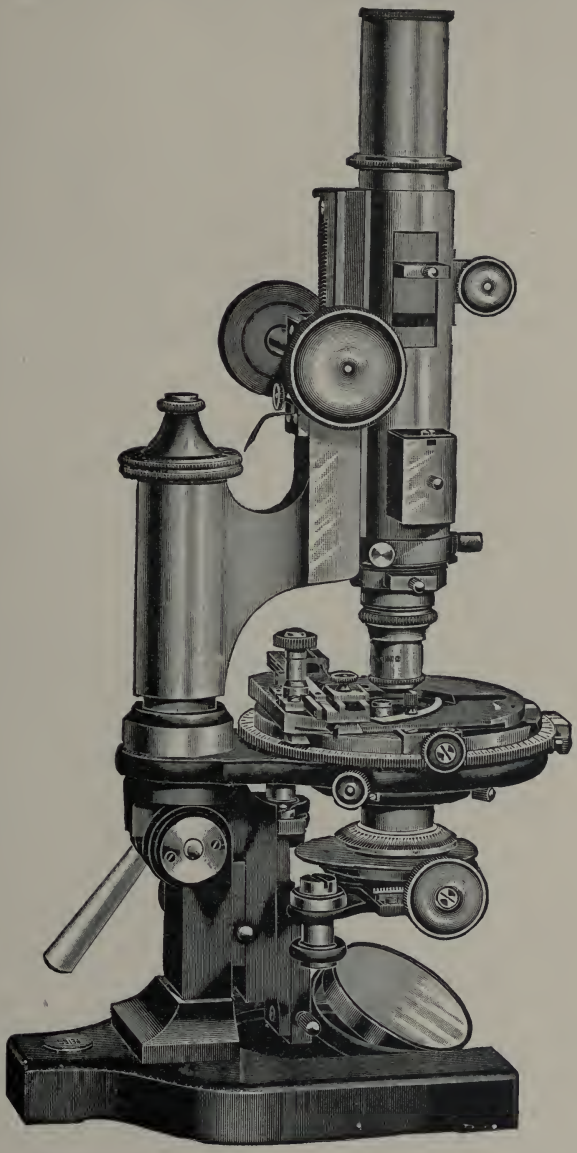


Fig. 175.—Petrological Microscope by Bausch & Lomb.

prisms. The polarising prism is carried in a revolving mounting, with pointer and silver circle graduated to 360°. Directly above the prism is a divisible condenser for convergent light. By an ingenious device the upper

lens of the condenser can be instantly thrown to one side without disturbing any of the adjustments. An iris diaphragm is mounted below the polariser to modify the light.

The polarising prism is carried in sliding prism box in the main tube, so arranged that it can be thrown into the optical axis of the instrument when polarised light is desired, or thrown to one side when the microscope is used in ordinary work.

Herr Leitz was of opinion that the many requirements for a perfect petrological microscope demand an instrument of larger size than hitherto constructed for the purpose. Hence his latest model—suggested we believe by Dr. Lincio and shown in Fig. 176—has been specially designed of a much more massive and solid character than any of his previous instruments. The boldly contrived upper portion affords more room for the manipulation of specimens which may be of very considerable size, and moreover the arch itself affords a convenient handle by which the microscope can be carried about without risking any injury to, or straining of, the fine adjustment as hitherto was likely to occur in the former type of stand.

No change is made in the arrangements for the coarse movement of the tube, but the new type of fine adjustment lately devised by the firm takes the place of the older form more commonly met with.

The tube and its draw-tube are very large in diameter. This is a convenience when using the instrument for the purposes of photography, as it thereby prevents any curtailment of the field of view when low powers are employed. Further, it affords —we cannot deny—more room for a better designed fitting to hold the analyser with its circle divided to 360°, enabling the various measurements carried out by its use to be more easily and efficiently effected.

A Bertrand's lens slides into the tube, the carrier made to hold the same being capable of removal when the lens is not required to be in use. See page 219, footnote.

The nosepiece, of a very solid character, is provided with centring adjustments and the usual universal thread.

The instrument has a large revolving stage, the upper plate being divided by rulings to facilitate the fixing in position of slides on the stage, centring screws being added for bringing into adjustment the whole arrangement so as to obtain perfect alignment with the optical axis. The ordinary rectangular

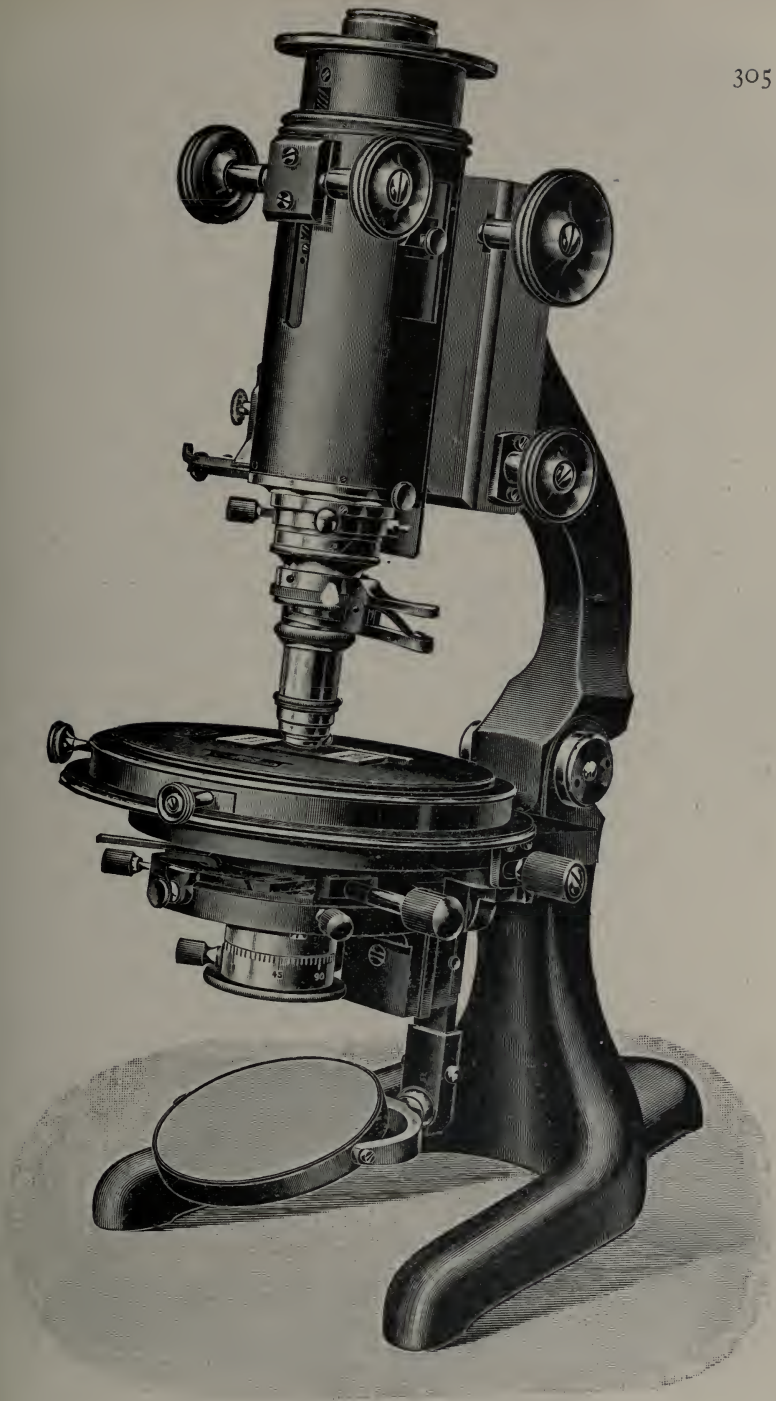


Fig 176 —Petrological Microscope (new model) by Leitz.

movements are present, and verniers, divided into millimetres, are attached to different parts of the instrument so as to record the position effected by any of these three motions mentioned.

The substage arrangements are modifications of the usual form, but a fine helical wheel-rim is placed beneath the stage that can be made to engage itself at pleasure with an endless screw, so as to assist the operator in obtaining the exact adjustment of an object during any operation involving goniometrical measurements. We have not been able to find that there are adjustments for the substage condenser, which we hope can be easily added.

Swift & Son, too, have paid much attention to their petrological stand illustrated in Fig. 177, which has been built after the design of Mr. Allan B. Dick.

This instrument differs from most petrological microscopes hitherto constructed in having a fixed stage. When revolution of the object is required, the same *effect* is produced by revolving both prisms and eyepiece *en bloc* instead. The spider lines in the eyepiece therefore turn on the object which remains stationary, so that the most minute specimen is maintained in position during the entire rotation, and hence the necessity for delicate centring adjustments to ensure accurate concentricity of revolution is altogether obviated. A divided circle rotates simultaneously with the prisms.

A converging lens is fitted over the polariser, and another, mounted in the slide A, can be combined with it when greater convergence is required. G and H are two horizontal slides in the optic tube of the microscope, each of which is furnished with a lens and a clear aperture. The lower one, G, is for showing rings round the optic axis of crystals ; and the upper one, H, is for exhibiting optic images in very minute crystals. Both have adjustments in the vertical plane.

The stage is divided to millimetres in both directions for recording the position of an object. E is a slot for the insertion of a micrometer, undulation plate, or quartz wedge: the two latter can also be used over the eyepiece. The fine adjustment is a differential screw motion, with milled head divided into 80 parts, each division being equal to 0·01 mm., thus enabling it to be used for finding the refractive index of any transparent mineral. The polarising and analysing prisms may be revolved independently of each other. The polariser is mounted upon an arm which allows of its being turned out of the axis of the microscope.

A Klein's quartz plate is made to drop into the open aperture of the slide G, and it can also be used in a holder on the stage.

An analysing prism can be mounted below the slide G (which can be readily thrown out). For those who require to photograph slides under observation the analyser fitted as above would be of considerable advantage, the one fitted over the eyepiece not being suitable for the purpose.

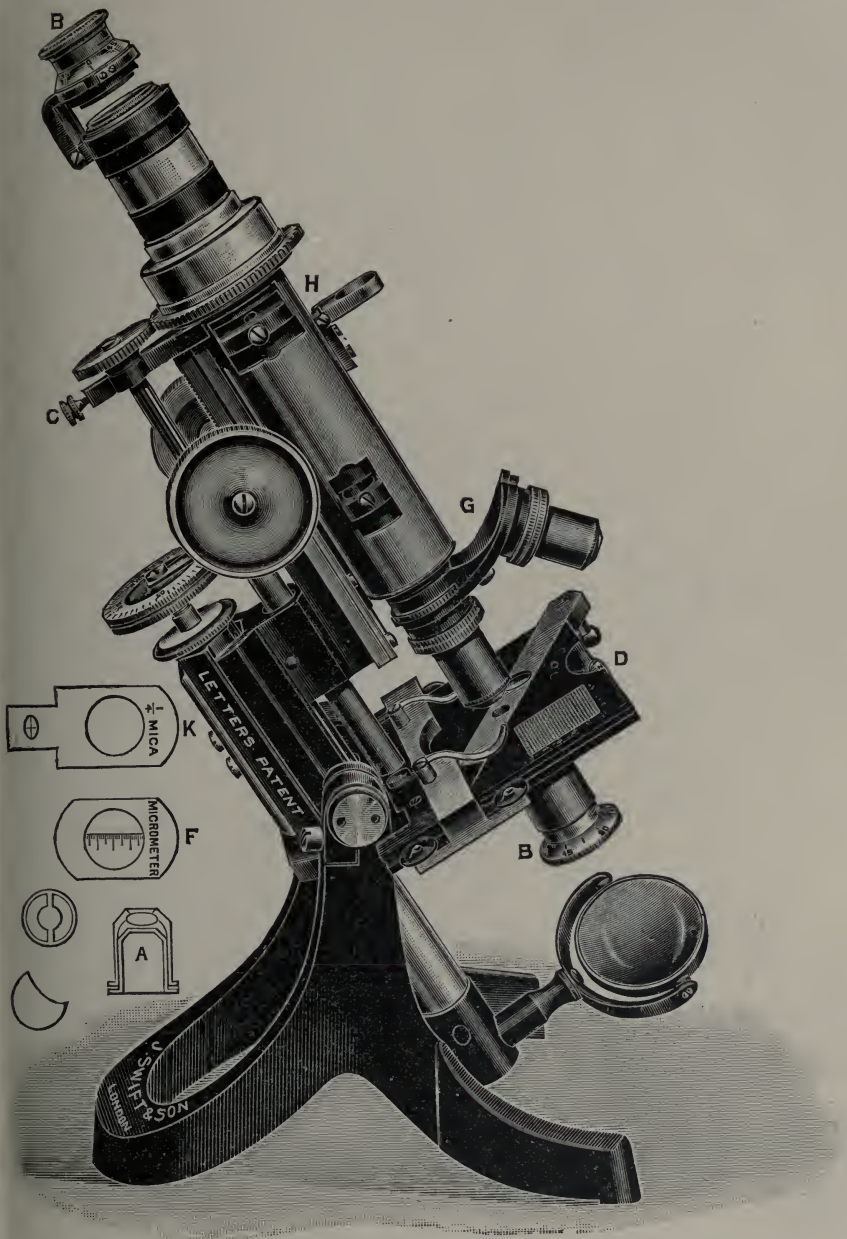


Fig. 177.—Petrological Microscope by Swift (Mr. Allan Dick's Design.)

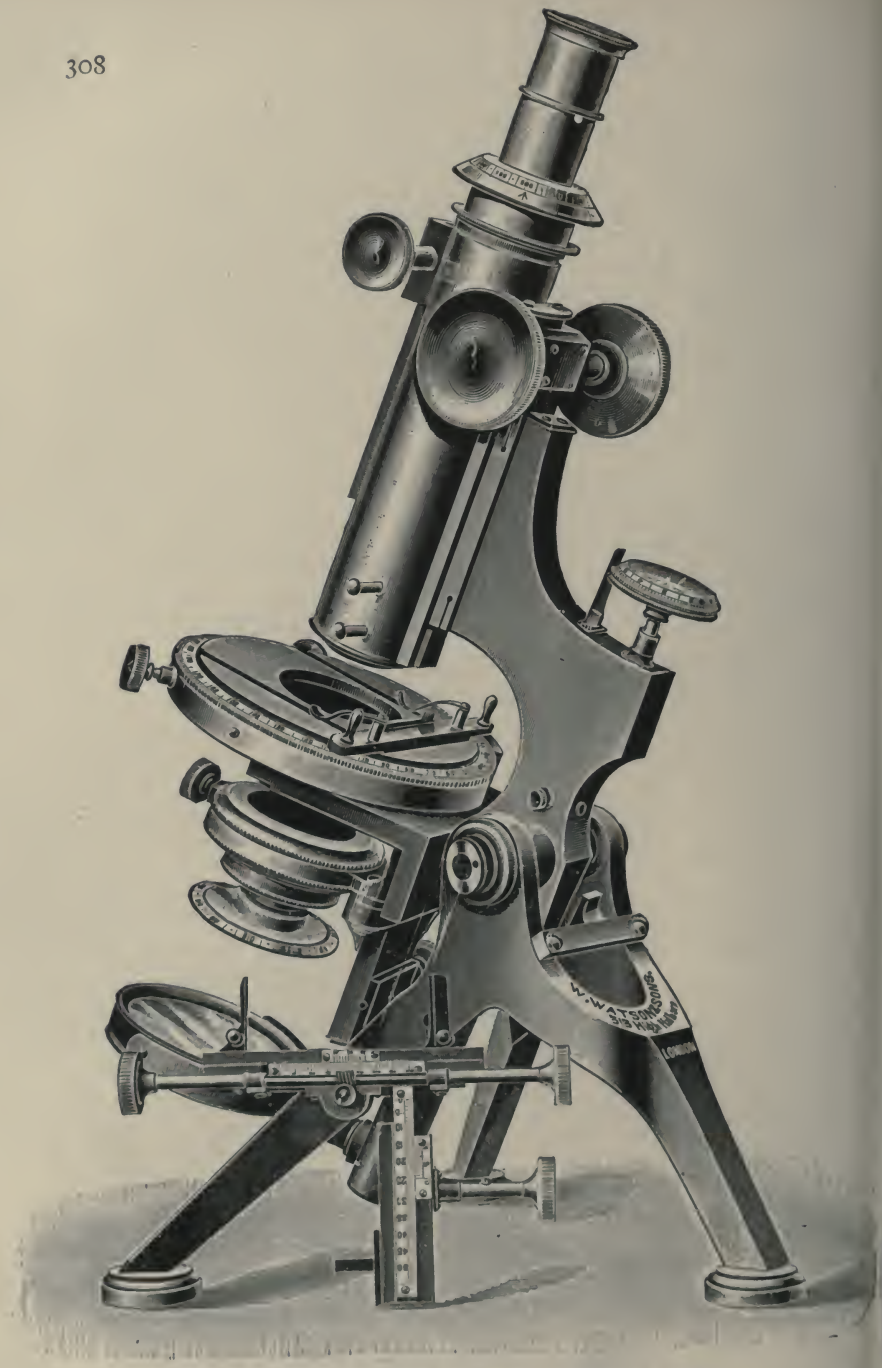


Fig. 178.—Watson & Sons' *Advanced* Petrological Stand.

If the microscope is required for non-petrological studies the analyser is thrown out of position, the polariser removed, and the achromatic condenser A inserted in its place. The condenser is furnished with an iris diaphragm and two stops—one for oblique and the other for dark-ground illumination.

Messrs. Watson & Sons have constructed of late years what they call an *advanced* petrological stand (Fig. 178), which must not be overlooked. In this instrument everything is excellently arranged, and the various contrivances to meet the requirements of the most critical users are carefully looked after.

The stage is a circular brass one, finished dead black, having a sliding bar to carry the object. It is divided to millimetres in horizontal and vertical directions. A separate mechanical stage, as figured, with divisions, and giving a long range of motion, is included, and can be attached at once when required. The circumferential edge of the stage is divided to degrees, and a vernier is fitted reading to five minutes. The stage rotates concentrically completely, and centring screws are provided for adjustment to the axes of different objectives.

The substage has screws to centre, rackwork to focus, and can be lifted aside with the apparatus contained in it upon a hinged joint; when set in position it is securely fixed by means of a clamping lever.

The body is provided with one draw-tube actuated by rackwork. At the lower end of this tube the universal thread is fitted to carry a Bertrand's lens, etc. At the lower end of the body is a Klein's quartz plate, and above it an analyser fitted in boxes, which can be instantly withdrawn when not required.

The eyepiece is provided with cross webs, and above it is fitted a rotating circle divided on silver reading against a fixed bevelled circle at the top of the draw-tube, and carrying an accurately adjusted analyser prism and calcspar plate, which may be used either separately or in combination. A polariser having a specially large prism is included, the rotating circle of which is divided on a silvered edge, each quarter-circle being indicated by a spring catch. A removable condensing system of high angle is fitted above the prism for showing brushes in crystals, etc.

The Microscope for Metallurgical Use

The study of Metallurgy by the aid of the microscope has been increasing very rapidly indeed of late; so much so that the investigations by Sorby about 1864, Martens in Germany about 1878, followed by Stead, Roberts-Austen, and others, have raised what might have been called in olden days nothing but an interesting study into an exact and far-reaching science. So much is this the case that within the last ten years consider-

able inventive skill has been brought to bear upon the subject of furnishing an instrument which would meet the requirements peculiar to the study in question. Of these there are several. For instance, it is requisite, of course, to use reflected light, because the specimens are necessarily opaque,[1] and this can be only perfectly effected by the employment of what is called the vertical illuminator, already described. But this very little piece of apparatus is itself a troublesome arrangement to deal with. Attached to the nosepiece of the microscope at one end, it receives the objective in the other, the light from the illuminant being projected into it through a small hole (guarded by one or more diaphragms) in its side. Arranging an auxiliary bull's-eye condenser to throw the beams of light *exactly* into this little hole just mentioned (from which it is reflected on to the specimen) is in itself a somewhat troublesome and fidgety affair; hence, when once set in order, the tube of the instrument must not be touched.[2] This forbids the use of the coarse adjustment; hence, as the microscope cannot be lowered to the specimen, arrangements have to be made to raise the specimen up to the objective, which means the construction of a special form of stand altogether different in build from any of which we have spoken. Then, to use the vertical illuminator successfully the objectives have to be specially made in very short mounts, so that the back lens of each shall be quite close to the illuminator. In addition, provision has to be made not only for the examination of *slices* of metals, but of sensible-sized pieces of material. Other details have also to be considered, so the result is an instrument that is highly complicated. That devised by Martens some ten years ago seems to have satisfied every requirement possible save those of certain faddists, and is used indeed in a very large number of technical colleges. Quite recently, however, Messrs. R. & J. Beck have constructed the "Rosenhain" model, which is said to have improvements of

[1] Occasionally, for exceptional reasons, plates of metal may be ground so extremely thin as to transmit light—anyhow, through certain parts—in which case they can be used on an ordinary instrument.

[2] Messrs. Watson & Son's have recently made a new form of vertical illuminator which meets this difficulty of focussing after the light is adjusted. It has a self-contained electric bulb, a condensing lens, and a bull's-eye. See page 209.

great importance; but concerning these we have not space to discuss, although we are bound to admit we are not persuaded of the utility of one particularly mentioned, that of the "permanently fixed tube," for it is obvious such large pieces of metal cannot be as easily accommodated as they would be in the Zeiss model after Martens, where it can be additionally raised to a considerable extent. Seeing this has a special screw to fix it when everything is set in readiness for use, so it cannot subsequently slip, what is the advantage of depriving the instrument of a more liberal adjustment? We also illustrate a very excellent stand made by Messrs. Watson & Sons, which, judging by the number of institutions they have supplied, seems to be a great favourite.

All these three instruments we propose to describe at some length as the subject in recent years is claiming so much attention, especially in the discovery of effects of sudden cooling, slow cooling, and medium cooling of steel, the arrangement of the ferric structure in ferrite, cementite, the properties of cast iron, showing how it differs from malleable cast iron, and the different qualities possessed by the various kinds of metals, such as molybdenum, chrome, tungsten, copper, and aluminium steels, let alone the signs of stress and fatigue in iron girders, and many other interesting subjects connected with metallurgy.

It should be mentioned that in the construction of the Zeiss model (Fig. 179) its adaptability *for photographing the specimen as well as regarding it visually* is particularly taken into account; hence many of the extra details relate more to this side of the question than they do with respect to purposes of simple inspection. We feel, however, a full description is necessary if only to compare with other stands.

In external appearance the stand differs greatly from the more usual forms. A square foot, which can be fastened by four screws to the sole-plate of the protection table or on any other suitable base, supports a massive horizontal rail. A carrier, to which the tube is attached in horizontal position, is screwed to one end of this rail. The tube is provided with only a coarse adjustment by rack and pinion T', and is principally intended for work by transmitted light. A slide, worked by a second rack and pinion movement T'', is situated at the opposite side of the rail; a lever H, on the lower side of the rail, gives facilities for securing this slide at any point in the course of its movement. This slide includes the bearing for a second, a smaller slide, which can be moved in the most exact manner by the micrometer

screw *M*. The head of this micrometer screw bears a divided scale, by which movements of the slide by 0·005 mm. (5 μ) can be immediately read off. The edge of the micrometer screw consists of a crest of oblique teeth into which a pinion *Tr* engages. This pinion can be set in motion from the position of the focussing screen of the camera by means of Hooke's

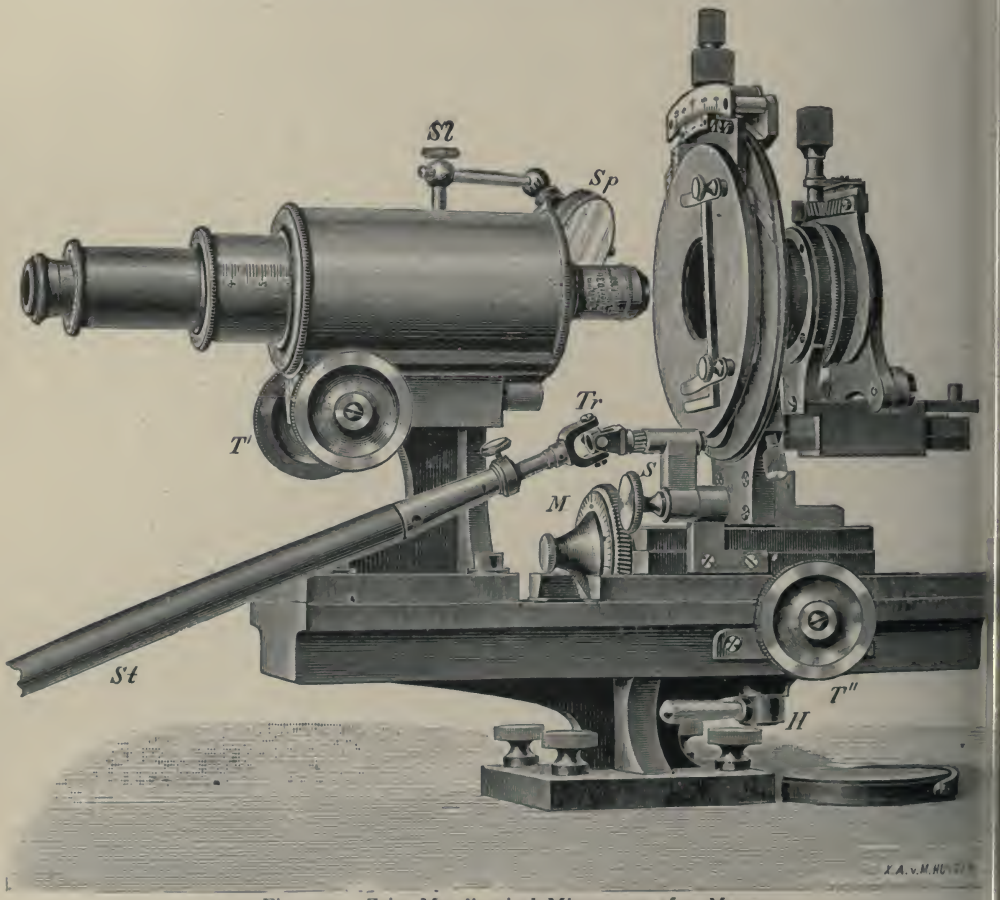


Fig. 179.—Zeiss Metallurgical Microscope after Martens.

key, which is joined to a removable wooden rod *St*. The pinion is connected with a revolving arm: when the micrometer screw is to be moved directly by hand, this arm is turned aside after easing the screw *S*. A special form of stage, suggested by the Royal Institution for Technical Research, Charlottenburg, can also be added to the stage (after removal, if necessary, of the Abbe illuminating apparatus). This appliance is fitted with adjusting-screws, so as to enable the surface of metalliferous prepara-

tions, held in position by spring stage clips, to be adjusted vertically to the optical axis.

The illuminating accessories for reflected light are attached to the tube, whose rack and pinion action for focussing the preparation is not required for this manner of illumination.

With higher magnifications the vertical illuminator is employed. For low magnifications, obtained by means of objectives giving sufficient object distance, either a plane or a concave mirror is used, or a thin plane-glass, inclined at 45° to the axis, is placed between object and objective so as to partially reflect upon the object the light falling vertically to the axis. The plane and concave mirrors are mounted together in a gimbal in the usual manner, so that they can be conveniently turned in any direction (Fig. *Sp*). The gimbal is joined to a rod, which is secured by a clamping-screw at the upper end of the small stem *Sl*. This stem is vertically movable in a cylinder—not seen in the illustration—and secured by a second clamping screw at any desired elevation. The cylinder itself is situated on a slide-screwed on to the tube parallel to the optical axis—which is also movable by hand and is secured by a third clamping-screw. In this manner provision is made for a very extensive movement of the mirror.

The plane glasses are attached in exactly the same manner as the mirrors. A large or small plane glass is to be used, according to object distance and extent of the field to be illuminated.

The "Rosenhain" Metallurgical Microscope

This microscope, as seen in Fig. 180, is built on quite different lines from the preceding.

The limb has been designed to constitute a properly proportioned truss. It has a **T**-girder section throughout, the upper front portion having the body rigidly attached, and the lower front portion having a wide dovetail fitting upon which a solid bracket holding the stage racks up and down. Besides, it is jointed on a centre so arranged that the instrument is almost in balance from the vertical to an angle of about 30°, and at the horizontal position for photomicrography it rests on a projection of the base, which forms a cradle to support it. Thus, it is claimed, there is no strain on the joint fitting when used for visual purposes and when horizontal for photomicrography. The limb is provided with a socket and clamp for holding bull's-eyes, etc.

The coarse focussing adjustment is by means of a spiral rack and pinion, raising and lowering the stage for a distance of $3\frac{3}{4}$ in. when the instrument is vertical, and a further 1 in. when the instrument is at a slight angle or horizontal. The fitting is $1\frac{1}{2}$ in. wide, and fits by means of a broad dovetail.

The fine focussing adjustment is by means of a micrometer screw which is situated in the optic axis, immediately under the object, where it is claimed to be of special utility, but for what reason is not immediately apparent.

The body consists of a very thick tube, at the lower end of which is a nosepiece for carrying the object glass with centring adjustments, by which

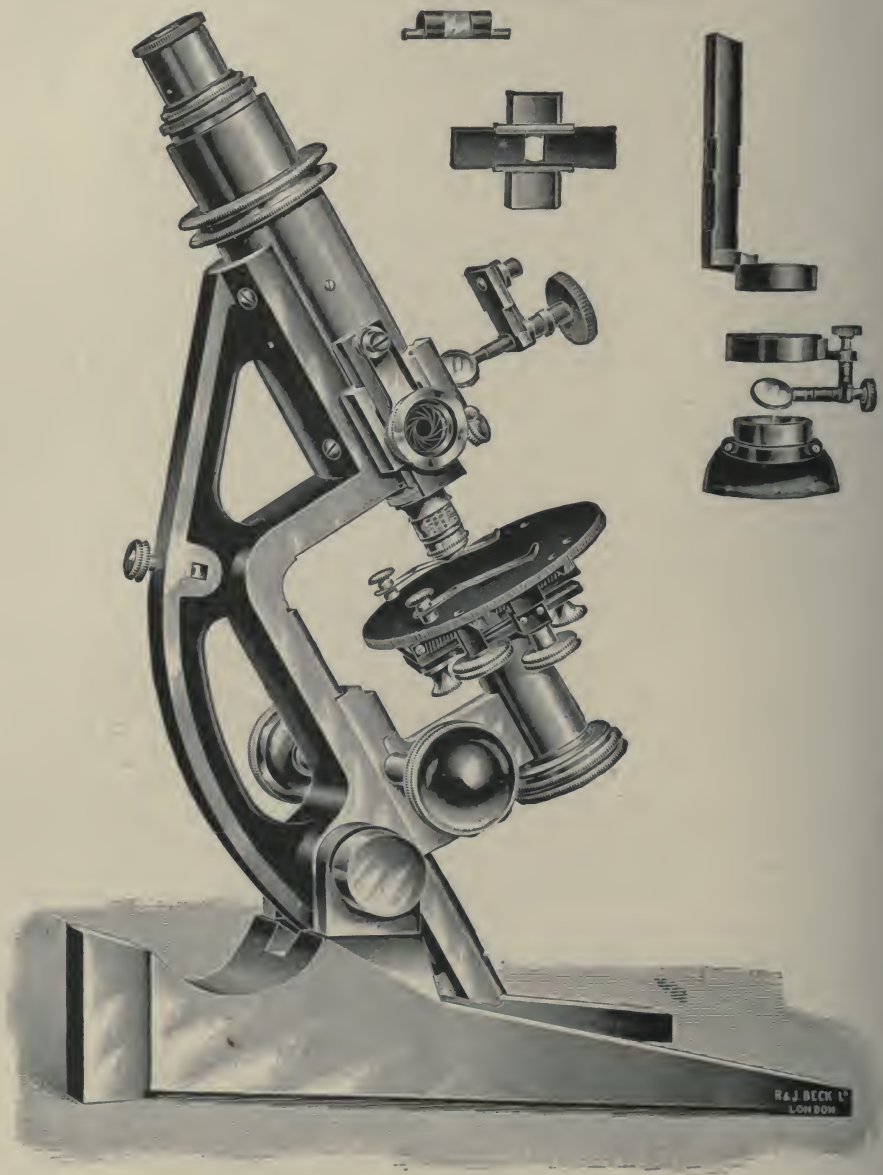


Fig. 180.—R. & J. Beck's "Rosenhain" Model.

different object glasses can be adjusted to the exact axis of the microscope around which the stage rotates.

An iris diaphragm actuated by a lever is fixed immediately behind the nosepiece, by means of which the aperture behind the object glass can be constricted for increasing its depth of focus or for cutting off reflections.

Three dovetailed fittings are supplied at the lower end of the body, into which all illuminating appliances and their diaphragms are attached to the instrument. These include various thin glass and prism illuminators for throwing light through the object glass. The parabolic speculum, the thin glass illuminator, and the Sorby's $\frac{1}{2}$-field flat speculum for use between the object glass and the object, also the coloured glass screens for simultaneously illuminating with light of different colours in different directions, an amici prism or bull's-eye for further illumination—all can also be attached by these fittings.

A supplementary draw-tube is provided for lengthening the body-tube when required. At the extremity of this tube a ring with the standard object-glass thread is provided, so that extra focussing adjustment can be obtained when using very low power or photographic lenses which might not focus in the ordinary way.

The stage has a complete rotation, which may be clamped for watching the effect of illumination at different angles and for placing the object in any required position. The centre of rotation is in the exact optic axis of the instrument, and any variation of different object glasses can be compensated by the centring nosepiece on the body.

Mechanical motions in both directions, by spiral racks and pinions of 2-in. travel, are provided in the complete form of the instrument, and they are so arranged that a complete rotation, which is always concentric with the optic axis, can be obtained when the motions are used for 1 in. of their travel. The adjustments of the stage are so designed that, with the exception of the stage springs, which can be removed, there is no projection to interfere with the illumination or cast shadows when a very oblique pencil of light is employed.

A transparent attachment is supplied for use with transparent objects with the same efficiency as that obtained with standard model microscopes. It fits above the level of the stage, and a supplementary table which bridges it over fixes on to the stage of the microscope and partakes of the mechanical movements and rotation possessed by the stage itself, while the illuminating apparatus remains a fixture, as the latter is fixed not to the stage but to the stage bracket. The whole instrument is well thought out and finished.

A parabolic Lieberkuhn, Sorby's silver reflector with special objectives are supplied, including a $\frac{1}{6}$-in. *oil immersion object glass* constructed, not for the purpose of giving an increased angular aperture, but to avoid reflections and to give greater depth of focus or penetration than is possible with a similar angle dry glass. This appears to be a very useful addition.

Quite a different class of instrument is made by Swift in two forms, the first being designed by Mr. J. E. Stead, F.R.S., etc., for use in workshops, and the other a Compound Metallurgical Microscope designed for the Royal Arsenal, Woolwich.

The first (Fig. 181) is arranged to be employed in engineering works where large forgings, etc., require examination *when in the lathe or when laid on the ground.* It is specially massive. A solid stage is made to swing round so that the object glass can be brought into focus on the steel or iron

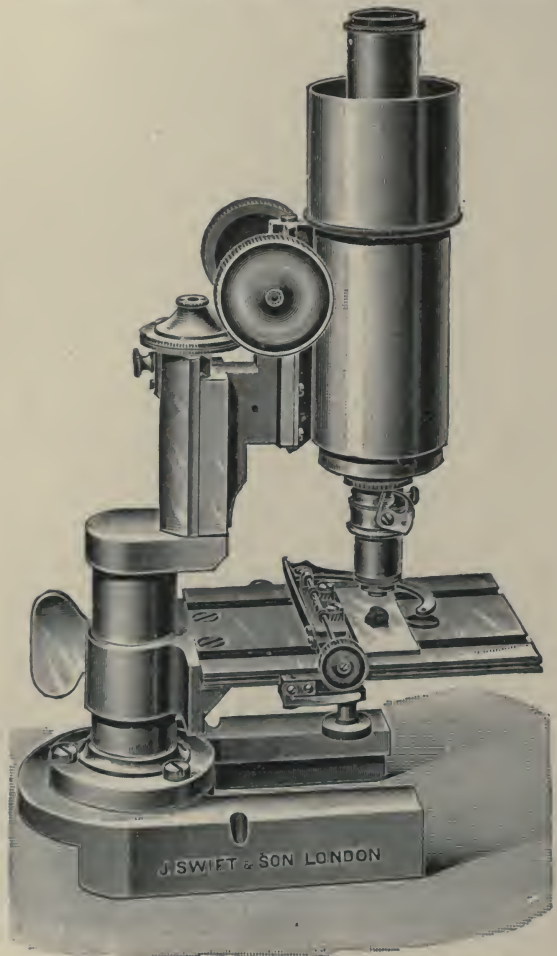


Fig. 181.—Swift & Son's Compound Metallurgical Microscope.

Fig. 182.

forging or casting, upon which the foot or fork rests. To effect this, an inside tube carrying the object glass slides within the outer barrel, and can be lowered to a sufficient distance. By means of a simple wire rope strap the stand is rigidly held in any position required on the massive piece of metal under examination. When in focus the position of the barrel is fixed

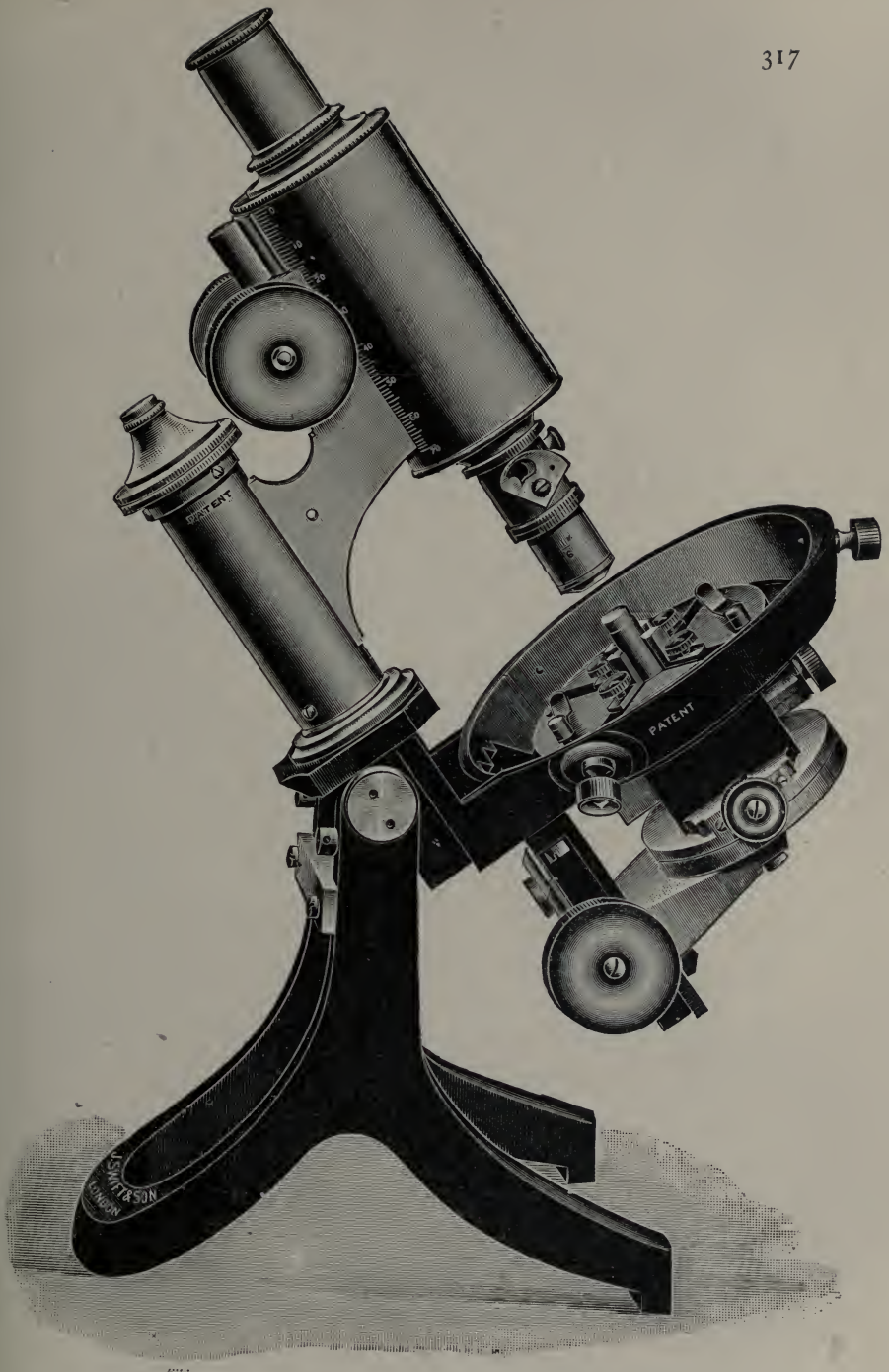


Fig. 183.—Swift & Son's New Compound Metallurgical Microscope.

by a screw at one side of the rack and pinion. When so fixed the conical camera (Fig. 182) is placed on top of the barrel, and a photograph can then be taken. When not employed in the fitting shop, the microscope can be used in the laboratory or office, and is suitable for all metallographic work.

The second variety (Fig. 183) is the New Compound Metallurgical Microscope originally designed for the Royal Arsenal, Woolwich.

The coarse adjustment is by spiral rack and pinion, and the optical tube $2\frac{1}{8}$ in. diameter; when the draw-tube is removed it allows of photographs being made of large or coarse objects with the smaller type of photographic lenses. The draw-tube takes the ordinary oculars. The slow motion is by the "Ariston" lever fine adjustment, described elsewhere in this book, and the stage is of entirely new and original design which admits of the object, after having been focussed in the horizontal position, *being tilted or turned in any direction*, so that the light impinging on the object from any source is maintained, thus enabling objects to be thoroughly examined at any point of inclination without being thrown out of focus, and so entirely doing away with the different types of supplementary levelling stages. This is effected by the base of the stage holding the part of specimen under examination moving in the segment of a circle or basin, a corresponding segment working it in carrying or holding the specimen, the point at which the object is viewed being the centre of the circle. The stage is fitted with mechanical movements for shifting the object in rectangular directions and the specimen can be entirely revolved round the optic axis of the microscope. It is also provided with a rack and pinion for moving the object up or down from the objective. The ordinary 3 in. × 1 in. slide is held upon the top of the stage by means of two steel springs which are removable. Pieces of metal, etc., are held in position by four clamping dogs sliding in dovetails and are held in position by small clamping-screws. The optical tube is divided to show the position at which any objective will allow of an object being tilted without going out of focus.

Metallurgical Microscope

By Messrs. Watson & Sons

A pattern of instrument differing in appearance from any of the preceding is sold by Messrs. Watson & Sons under the name of the "Works" Microscope. It is shown in Fig. 184. Made originally according to the specification of Mr. H. L. Heathcote, research student to Messrs. Rudge-Whitworth, Ltd., Coventry, it seems to have met with a very pronounced approval both in this country and abroad, for it has been supplied to such

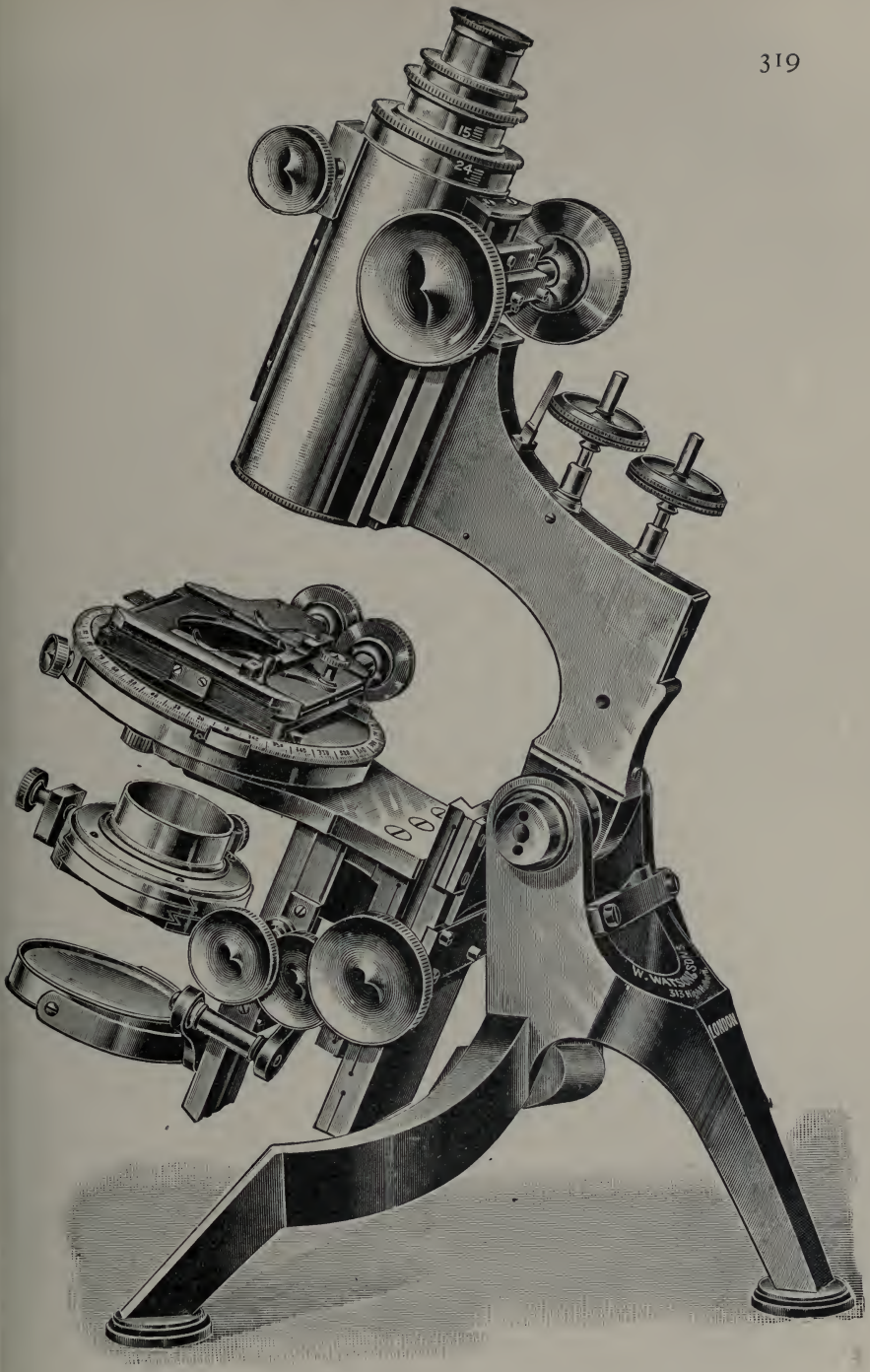


Fig. 184.—Watson & Sons' "Works" Microscope.

firms as Messrs. Armstrong, Whitworth & Co., Messrs. Vickers, Sons & Maxim, not to mention the Mint and several of the Railway Companies in England, whilst the Imperial Japanese Navy are in possession of two for their works.[1]

The foot is of the tripod pattern, which affords great stability no matter at what angle the instrument may be inclined.

The stage is mounted on a very substantial bracket, which at the back is fitted by dovetailed grooves into a frame, in which, by rack and pinion, it can be raised or lowered to or from the body of the microscope. It is usually supplied with mechanical motion and is provided with verniers. Complete rotation can be made so that specimens may be examined under every aspect of illumination. A sliding bar is fitted to a recess in the stage ; this bar may be instantly removed so that either a levelling stage or metal holder may be substituted.

All the necessary auxiliary apparatus are supplied with the instrument and means provided for their separate use ; whilst a substage with rackwork, a mirror, and other details are added, so that the microscope can be employed for examining extremely thin sections of metal or for any other purpose where transmitted light is used.

There seems no doubt that a considerable time must have been spent in devising and perfecting this arrangement, more especially in rendering it both a practical and portable one, devoid so far as possible of additions that may be regarded by some as superfluous and perhaps troublesome in everyday use.

Portable Microscopes

Of recent years the want of a microscope that was built especially for the purpose of being easily carried about has been much felt. This has originated, of course, from the fact that the instrument being now so much used in the arts and sciences may often be wanted when the user is away from home, and, as respects the medical profession, when the practitioner is on his rounds. Further still, the researches carried on upon the subject of malaria, and other diseases of a similar character which require investigation in remote parts of the world, demand the use of an instrument that is not a mere toy, but one of somewhat superior type. For this reason

[1] Since the issue of the first edition of this work Messrs. Watson & Sons have introduced a new horizontal form of Metallurgical Microscope which is well spoken of.

portable microscopes are of two classes, those suitable for low-power work, and those adapted for critical examinations. These in the following figures can be disseverated by mere inspection.

One of the most useful for the first purpose is shown in Fig. 185, by Leitz: we have employed one for years and can find no fault with it of any description. It is called a

Fig. 185.—Leitz's Hand Microscope.

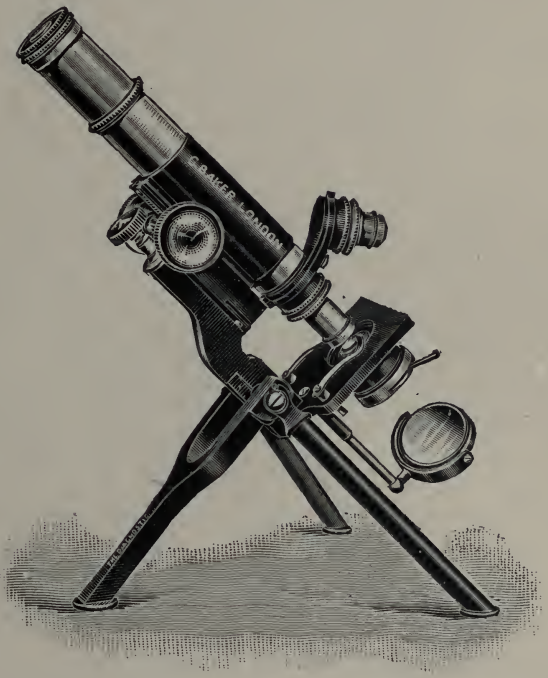


Fig. 186.—Baker's "Diagnostic" Microscope.

demonstration microscope. A chromatic Abbe condenser with iris diaphragm is provided, and a substantial handle so that the fine adjustment by means of a ring just above the objective can be easily actuated. Coarse adjustment by a sliding tube.

Another entirely different model is the "Diagnostic" by C. Baker (Fig. 186). Dimensions given below show how very lightly and consequently how very portably it is made.

The dimensions of this little instrument may be mentioned:

width of stage, $2\frac{3}{8}$ in. ; depth, 2 in. (from back to front) ; spread of feet, 7 × 7 in. Although exceedingly light this arrangement is far steadier than it looks. It folds into a very small case, $10\frac{1}{2}$ × 4 × 3 in.

One of the smallest microscopes ever manufactured is very

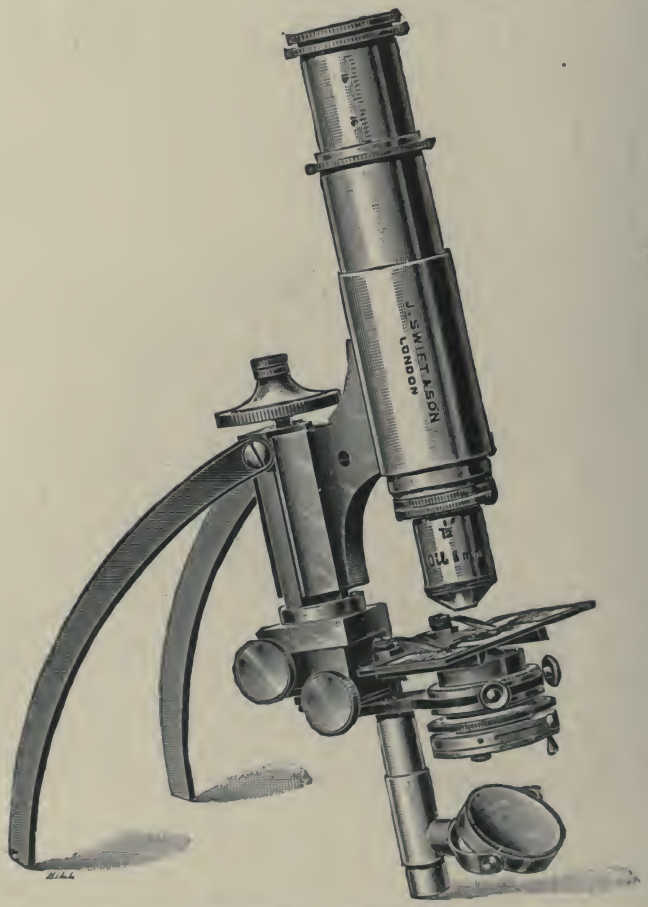


Fig. 187.—Swift's Small Portable Microscope.

probably that designed by Swift. Fig. 187 shows it open, and Fig. 188 packed for travelling. It has a mechanical stage and a centring substage, and altogether is an arrangement admirably suited to the medical practitioner, being fit for examination of

blood in malaria, anæmia, and inflammatory conditions. It may fairly be called a pocket instrument, as the case, made to hold both the instrument and bottles of solutions and reagents, blood-counting slide, pipette, and other details only measures 8 × 4 × $1\frac{3}{4}$ in. and weighs under 2 lb. If the microscope be

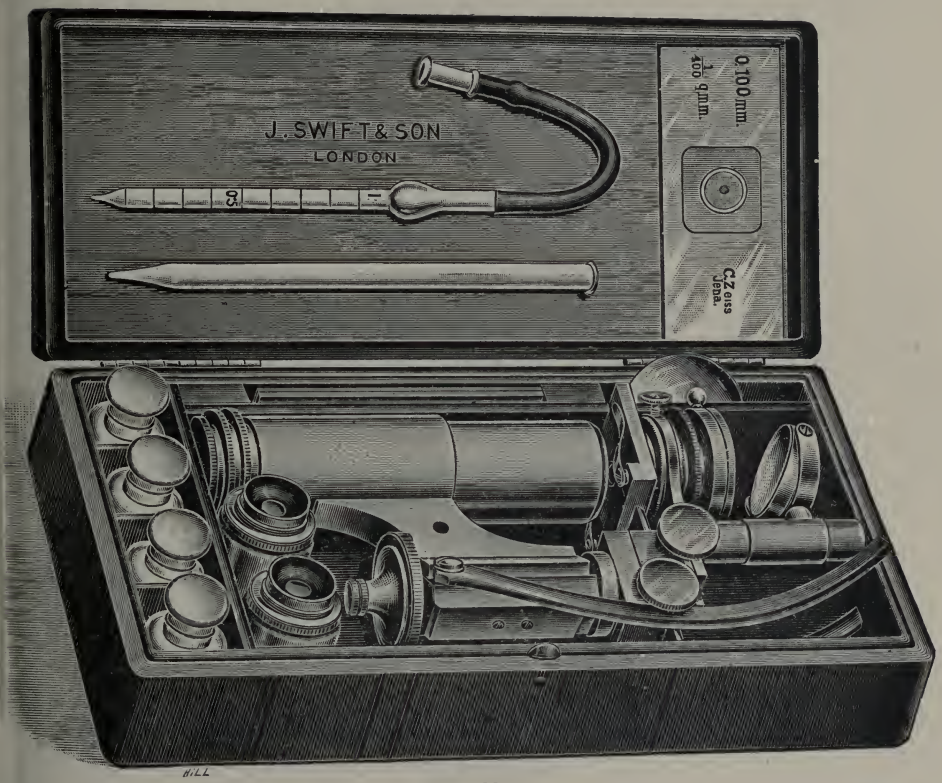


Fig. 188.

intended for use by the amateur, where the above additions are not requisite, the case only measures $6\frac{1}{2}$ × $3\frac{1}{2}$ × $1\frac{3}{12}$ in. and the weight with two objectives is under $1\frac{1}{2}$ lb. This is an arrangement we cordially recommend to the medical profession for all-round clinical work, where a more expensive instrument may not be desired, and where portability has to be mostly considered. We have with this arrangement used a twelfth

without difficulty, although we admit a certain amount of extra care has to be employed.

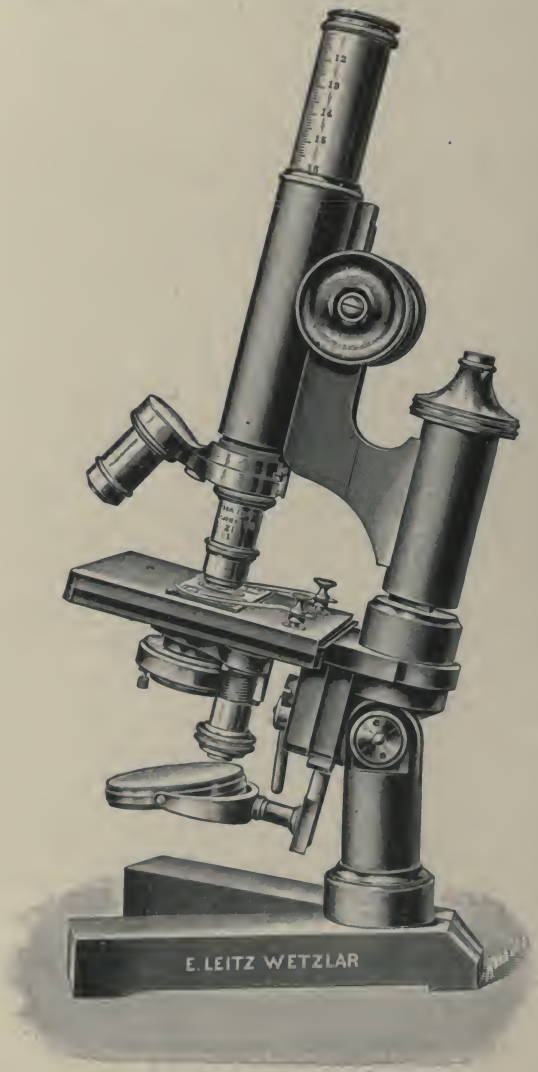


Fig. 189.—Leitz's Portable Microscope.

Of the more severe types of stand, one of the most perfect of the portable variety is that by Leitz (Fig. 189).

This instrument is suited for the most critical work, the case being constructed to hold several accessories. It is somewhat heavy; but, where weight is not of material importance, the advantage of greater steadiness is readily felt. It has a fairly good substage, but no mechanical stage, the makers relying upon the addition of the auxiliary form when necessity demands.

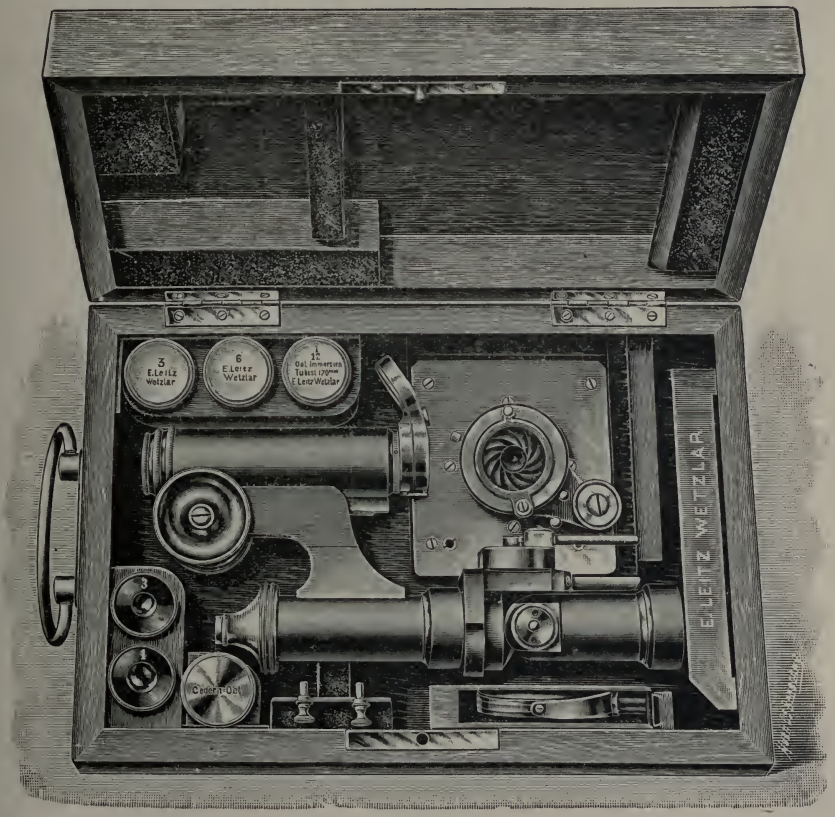


Fig. 190.

It packs away in a case, shown in Fig. 190, that measures $11 \times 7\frac{2}{5} \times 3\frac{1}{5}$ in. approximately.

Messrs. Watson & Sons have recently introduced a model they call their High-power Portable Microscope, which meets the special want of those who require an exceptionally strong instrument and one that can be employed when using high

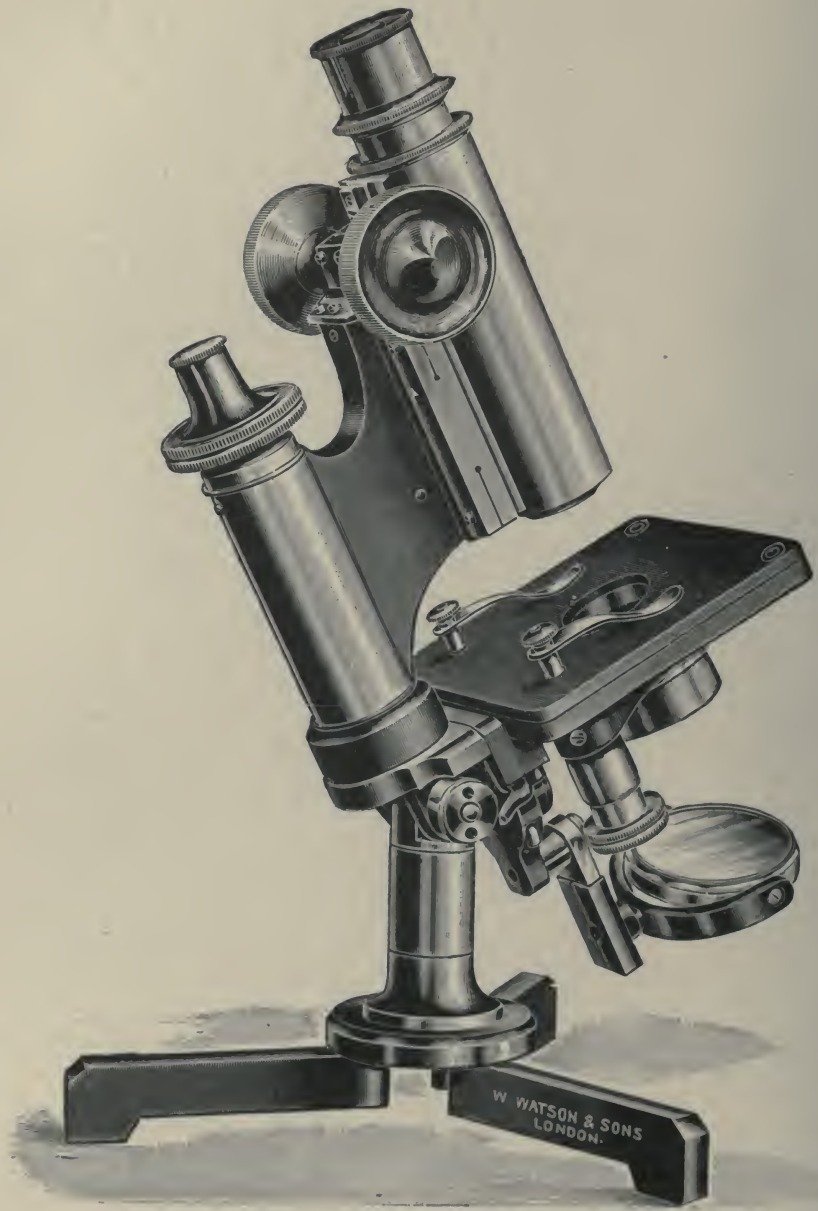


Fig. 191.—Watson & Sons' High-power Portable Microscope.

powers for really critical work. This is an ideal microscope, the makers say, for those who have to travel abroad, up-country perhaps, where travelling is heavy and the baggage may receive some rough usage, such as would imperil the safety of an instrument unless specially constructed for the purpose. It is shown set up for use in Fig. 191 and closed in 192, the case

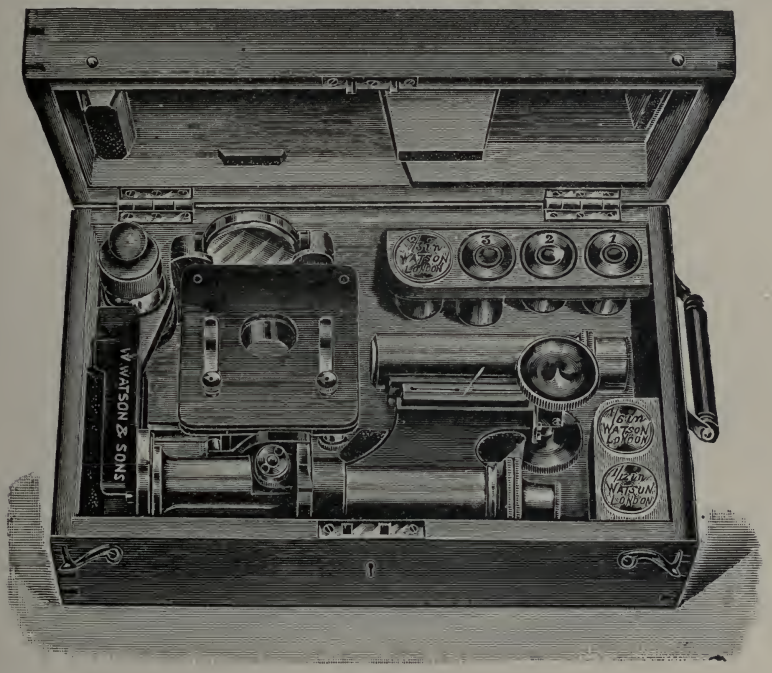


Fig. 192.

measuring only $11\frac{3}{4} \times 7\frac{3}{8} \times 4\frac{1}{8}$ in., and the total weight not exceeding 8 lb. 13 oz.

Lightness and rigidity such as to obtain a really useful and efficient instrument seem to have been the aim of the makers. The stage is of ebonite and measures $3\frac{1}{2}$ in. square. Substage condenser, with means of adjustment.

Several other opticians now sell equally good patterns, notably Messrs. R. & J. Beck and Reichert—limits of space only precluding the possibility of illustrating their excellent productions.

Semi-portable Instruments

The Museum Microscope

The idea of introducing this class of instrument is to provide for a museum a microscope that will show to a student a series of objects or portions of one object in succession, and one that is so arranged as to guard against the possibility of any accident, whether from ignorance or malicious intent. We mention two. The first used to be made by Leitz (Fig. 193).

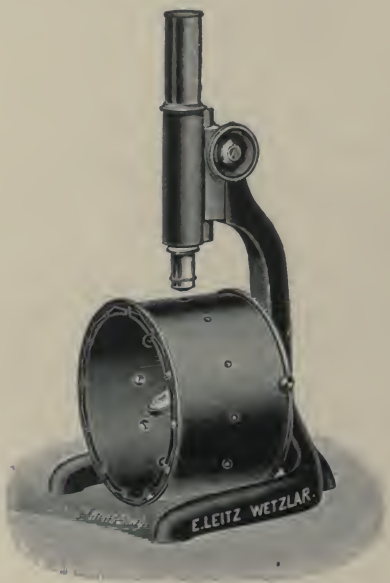


Fig. 193.—Museum Microscope by Leitz.

In this instrument the stage is replaced by a drum capable of rotation from left to right, and provided with supports for twelve preparations; the latter being separately retained in position by clips. Another detachable drum of sheet metal serves to protect the specimens from damage. Both drums are perforated by twelve apertures for illumination and observation. The interior of the drum contains a mirror which is movable in all directions. A spring register at the back of the drum ensures the correct position of each specimen as it comes under observation. The microscope is only provided with a coarse rack and

pinion adjustment and is accordingly solely adapted for use with low powers. The stand is supplied in a special case (not shown in the figure), which prevents the public from doing any injury, whilst allowing the free use of the instrument for studying the specimens.

The arrangement by Messrs. Watson & Sons called the "Waterhouse" model is shown in Fig. 194. It is perhaps more conveniently designed than the former, and is said to embody several important improvements upon all previous patterns. Under a dust-proof case, the twelve objects, mounted on the

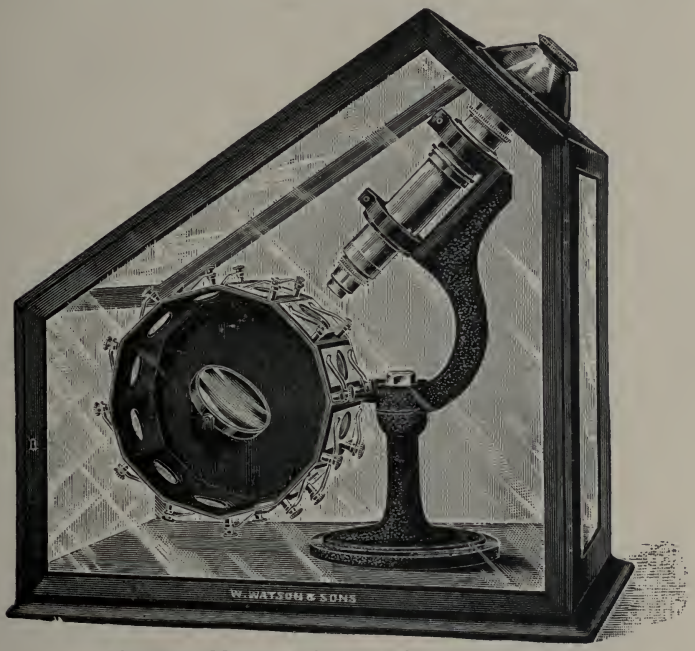


Fig. 194.—Museum Microscope by Watson & Sons.

standard 3 × 1 slips, are placed upon a revolving brass drum of very solid construction. The surfaces on which these objects rest are machine-planed, thereby ensuring proper focus for all the specimens when any change from one to the next is made. The drum is rotated by means of a milled head (not shown in the figure), so that the specimens can be changed without opening the case. Fine adjustment is effected by moving the eyepiece which projects well out of the case, and the back of the instrument is inclined at a convenient angle, as shown in the figure.

Microscopes for Critical Work

The class of instrument suited to the amateur, apart from the professional or business man, depends almost entirely upon whether his hobby is for low-power work, medium, or extreme magnifications. If his amusement and intellectual enjoyment are to be reaped from the vegetable kingdom, such as the study of botanical specimens and such-like, we have already said the objective and the instrument need not be of the very highest class or of the most expensive type ; but if, on the other hand, he elects to study fungi or such-like, he will require a fairly good installation, both as respects the stand as well as the objectives, and some of those we have already recommended will probably suit him. But, should his bent be towards the examination of pond life and other small objects in the animal kingdom, he may prefer a stand more of the portable type, or he may perhaps find a somewhat cheaper kind still is all that is necessary, as very high powers are never required for this class of work. As regards the objectives necessary, most authorities rarely employ more than three, a one-and-a-half, two-thirds, and a quarter. Of the first two, no more need be said ; but, as regards the quarter, the student will be well satisfied with what he obtains from Watson's Holoscopic series, Swift's best type, and Zeiss's achromatics, as they are all of excellent quality, as well as those by Leitz, Reichert, and Koristka, for they leave nothing to be desired. Baker's new two-thirds is a fine lens.

For the selection, identification, and mounting of diatoms a $\frac{1}{4}$-in. and often an objective of still lower power are mostly selected. When we come, however, to the study of minutiæ of any kind, whether it be of the animal or vegetable kingdoms, but especially with respect to the secondary markings of diatoms, nothing short of the finest possible stands and the finest of objectives are demanded. *It is critical work of the highest order.* It will be necessary, therefore, for us now to consider very carefully these first-class stands, after which we will discuss the finest objectives made, those which are called "the apochromatics."

It is but a fair remark to make that the typical English stand (Fig. 29), which we believe has been before the microscopist for a very great number of years—viz. that by

Messrs. Powell & Lealand—although designed so long ago when objectives were not made of such high power as at present are in *daily* use, still fulfils the requirements demanded by microscopists up to the present moment! One would be tempted to say the designer lived before his time! Handsomely made throughout of *hand-finished brass*, it is necessarily extremely expensive, and consequently out of the reach of many. One of the earliest of the Continental models, so different in aspect from the former, is shown in Fig. 195. Though others may equal, none could surpass the make of this firm (Carl Zeiss). We had two of their earlier models in very constant daily use for several years, and we have yet to find any fault with their performance. The figure shows the latest improvement, which is especially seen in the handle for lifting, which removes an objection to their previous designs. If the firm would arrange an extra draw-tube to enable the instrument to be used for "long-tube" objectives it would be a great convenience, and we regret the absence of centring adjustments in the sleeve of the substage condenser, for it is a serious omission.

Several additional stands, well designed and beautifully finished and generally of the very highest order of merit, are also made by other firms. One by C. Baker is called the "Nelson" model, and claims considerable attention as being designed by one of our greatest living microscopists who as a mechanician is only excelled by his adroitness as an observer.

It is essentially a universal form of stand, for it can be used for all low-power work as well as for the most critical; besides, it is so arranged that both long and short-tube objectives can be used with it as well as very *extremely* low powers—a complete combination not too often met with. There is one noticeable feature in its construction we have never met with elsewhere, and that is the fine adjustment is placed at the lower end of the body instead of in the ordinary position, as is seen by the most casual inspection of Fig. 196. Every movement is present in the mechanical stage with complete rotation, but any screws for centring the rotating stage should it by wear become eccentric do not seem to be present, which seems an oversight.[1] The substage is complete with a

[1] We learn that in recent models these have been added.

Fig. 195.—Zeiss Stand I.

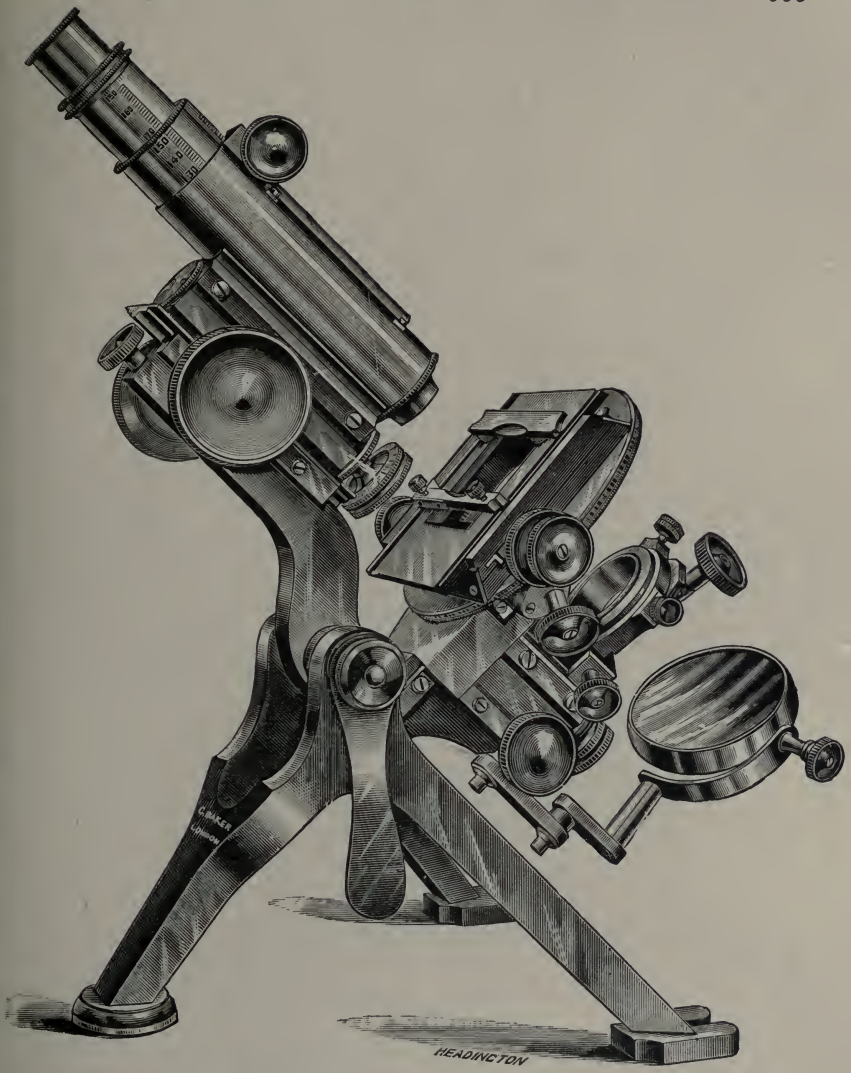


Fig. 196.—C. Baker's "Nelson" Model.

fine adjustment. A tripod foot is usually supplied, although a horseshoe can be substituted if desired. We know this stand gives the greatest satisfaction.

The American firm of Messrs. Bausch & Lomb, Rochester, N.Y. (Agents: 37-38, Hatton Garden, London, E.C.1), sell

a most magnificent first-class instrument (shown in Fig. 197) called "Stand CD." It has every adjustment we could possibly wish for, *including* a centring arrangement to the substage, although we regard this as the feeblest part of the instrument. This model has a novelty in the centring screws to the stage, which are provided with micrometers so that the extent of their movement, once ascertained to centre the stage in the optical axis, *can be registered for further use*; hence the stage, once carefully set, can in the future be readjusted without any further trouble. This is a most useful addition. If any alteration were contemplated, we should prefer smaller milled heads to the stage screws—especially that for transverse movement, as its large size we look upon as very inconvenient; but of course this is a most trivial objection. We should like, too, the adjustment for the substage condenser improved.

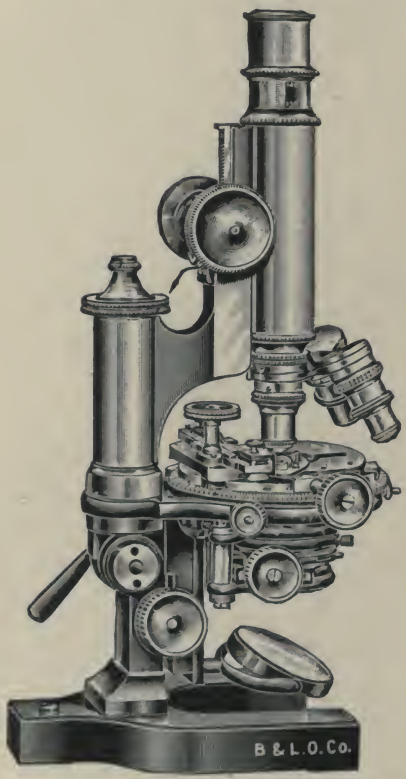


Fig. 197.—Bausch & Lomb's "Stand CD."

Beck's "London" Microscope ("Iris" model, Fig. 198) is a good piece of work. The stage is 4 × 4 in. and surfaced with ebonite, four clip-holes being provided. The mechanical stage is removable, which we have said before we object to save for bacteriological purposes. It has a travel of 2 in. horizontally and 1 in. laterally. An extra iris is contained in the substance of the stage, being placed there to avoid any accidental injury. The body can only be extended to 200 mm. (about 8 in.), so a lengthening tube would have to be added for the instrument to be used with English objectives of the long-tube correction.

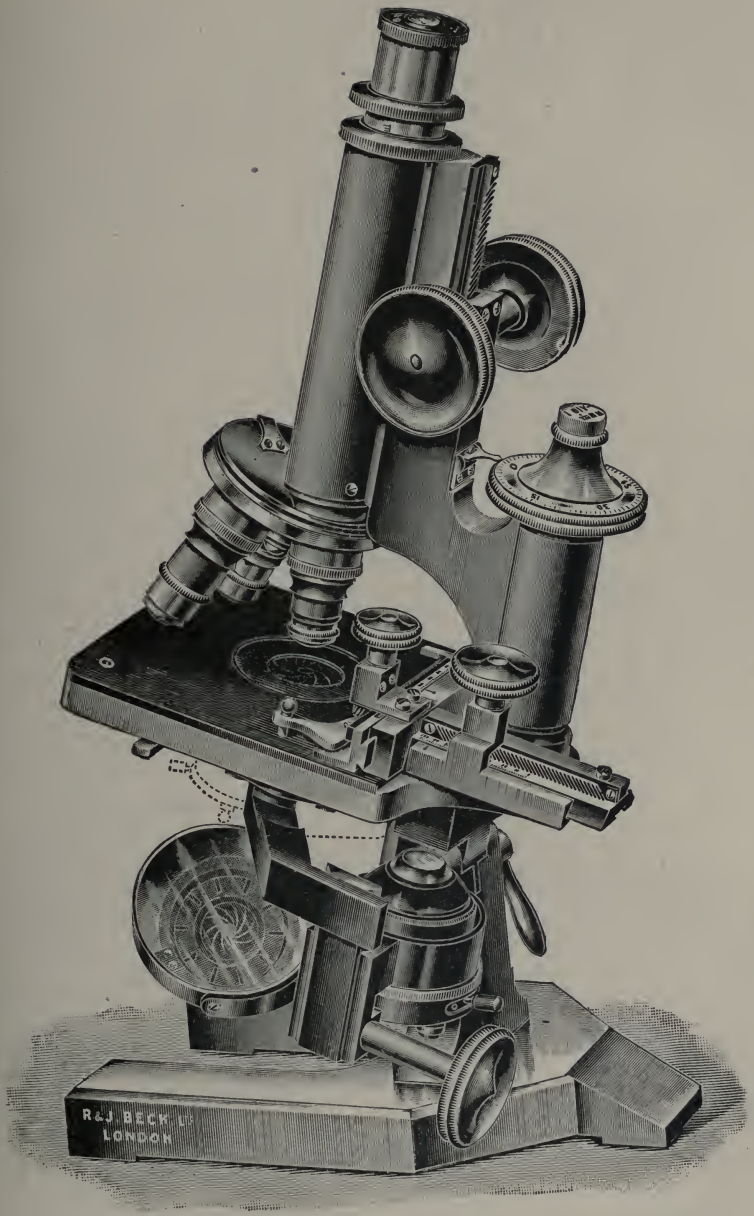


Fig. 198.—R. & J. Beck's "London" Microscope ("Iris" Model).

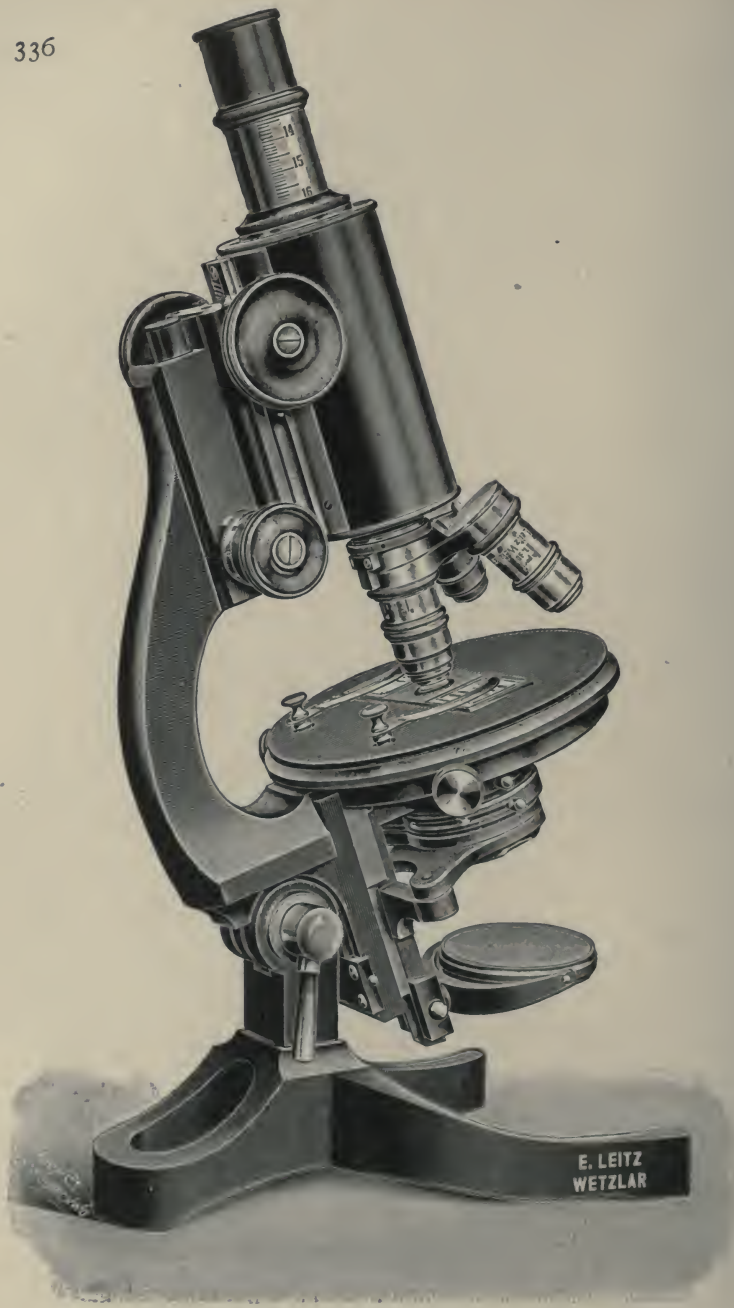


Fig. 199.—Leitz's "Universal Microscope Stand I."

No centring screws are provided to the substage condenser so far as we are aware.

The "Universal Microscope Stand I," by Leitz (Fig. 199), is provided with his new fine adjustment already described, has a standard type of revolving centring stage provided with a removable mechanical one, and is arranged for the short-tube work only, and so would need a lengthening tube when using English long-tube objectives. No centring screws to substage condenser. This stand is a practical one and a favourite with many, but for the highest type of instrument we should like the firm to arrange a model where the stage is built into the framework and an arrangement provided for centring the condenser.

Reichert's Stand No. Ia (Fig. 200) is an entire departure from the usual Continental model, the body taking the form of a handle, which so many opticians are now recognising to be of great service to the practical microscopist. In this instrument, as in the new Zeiss model, the use of the handle in no way threatens the safety of the fine adjustment. A circular centring stage is provided, and the firm's new fine adjustment, but no centring arrangements for the condenser. It is constructed for the short-tube objective only.

The Spencer Lens Co., of Buffalo, N.Y., have also an exceedingly fine stand of entirely new design, which is believed by them to be far superior to any of their previous make. It is shown in Fig. 201. It is exceedingly steady also, being well balanced.

Swift & Son's Portable Histological Microscope (Fig. 202) is of great excellence. Although primarily designed for the study of Histology, it is perfect as an all-round instrument. It has the peculiar feature first of having four legs, and secondly of being collapsable, for it can be packed away in the smallest amount of space imaginable, although a little practice is necessary to do this, as a study of Fig. 203 will lead the reader to understand. It may therefore claim admission into the class of Portable microscopes. Constructed for the short tube, it can be extended for an optical tube-length of 10 in., which makes it just a trifle too short for the convenient and scientific use of long-tube objectives, without an additional draw-tube or the use of Zeiss "sliders" or a revolving nosepiece, either of which just increases the length sufficiently.

Messrs. Watson & Sons' "Royal" and "Club" Microscopes are

Fig. 200.—Reichert's Stand No. Ia.

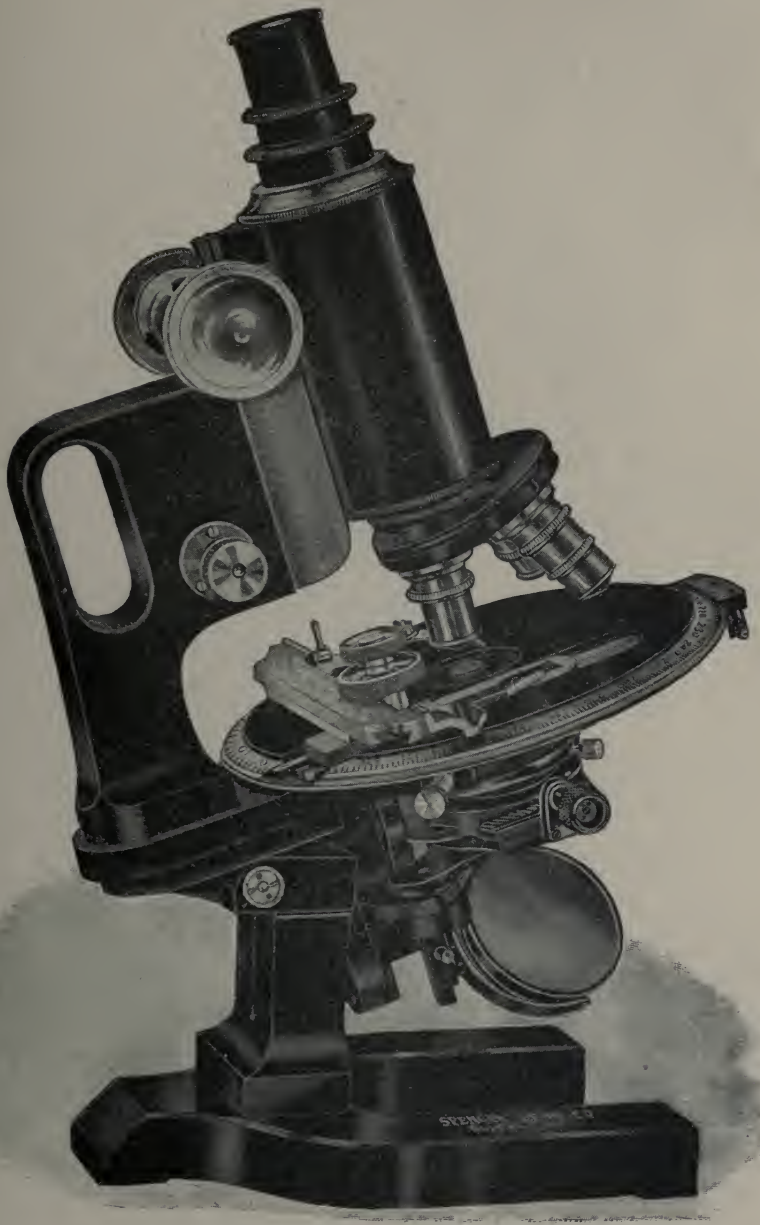


Fig. 201.—The Spencer Lens Co.'s Stand No. 10.

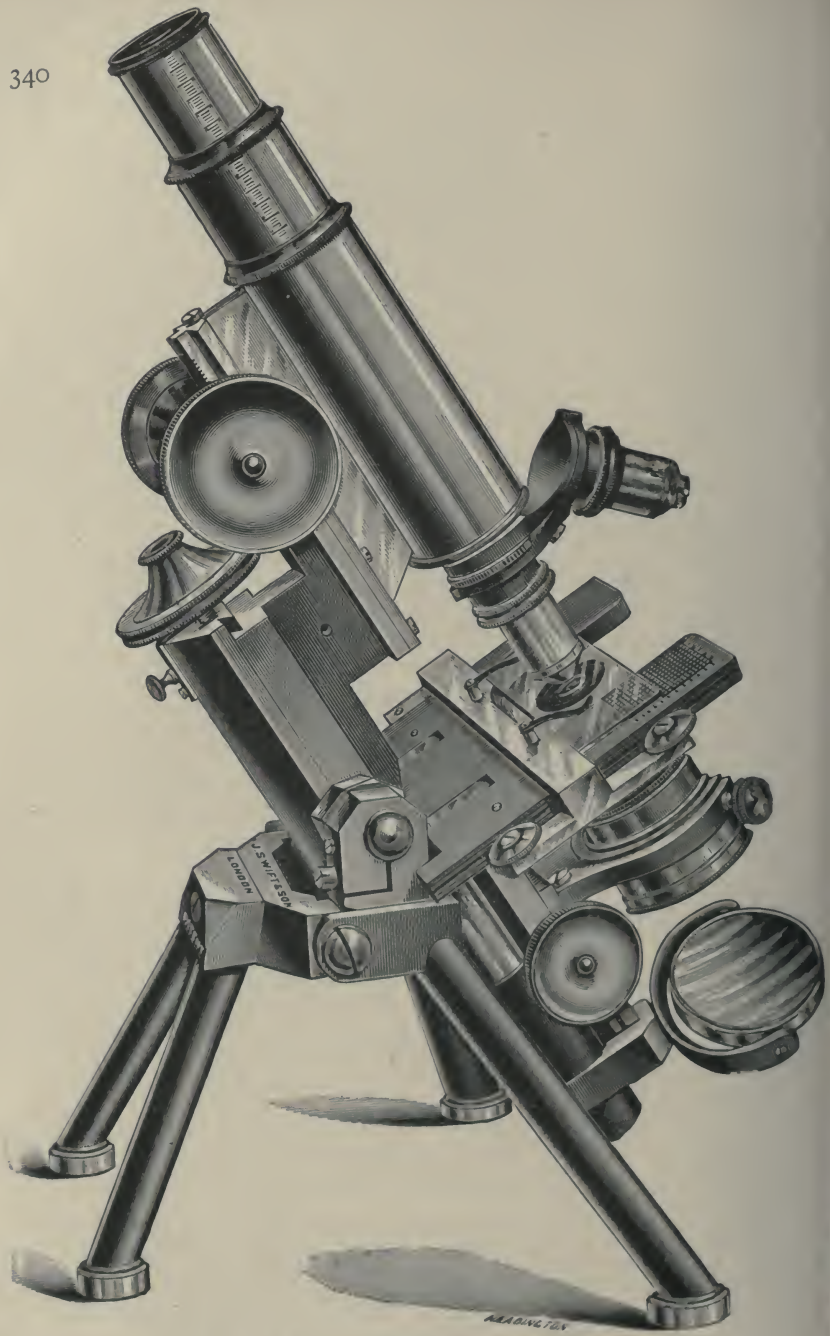


Fig. 202.—Swift & Son's Portable Histological Microscope.

to our mind of a more convenient pattern than their Van Heurck. They are both excellent models, being much alike. We describe the "Royal" (Fig. 204). It is a remarkably well-made instrument, being of very perfect construction, and, as the makers say—and this we can easily believe—"the outcome of numerous experiments." The fine and coarse adjustment are of the usual type supplied to the Van Heurck. The body tube is of large diameter, taking the English form of eyepiece (1·27 in.). Two draw-tubes are provided (one having rackwork), so the instrument can be used with both Continental and English form of objective, seeing it

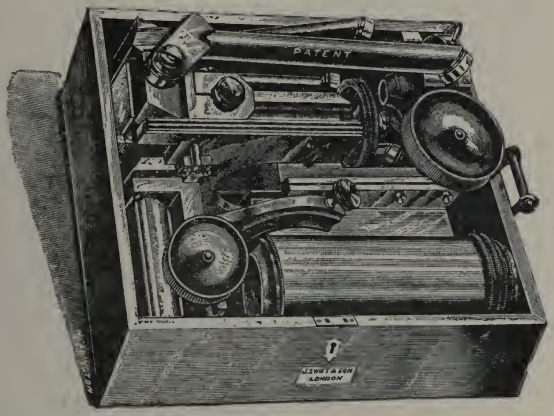


Fig. 203.—Microscope shown Folded in Cabinet (size, $7\frac{5}{8}$ × $6\frac{1}{4}$ × $4\frac{3}{4}$ in.).

closes to 142 mm. and extends to 305 mm. Centring screws to the substage are provided. This we believe to be a practical instrument and to have justly obtained considerable acceptance.

With respect to **the objectives** used for the highest power critical work, there is no doubt the apochromat leads the way. We have spoken already of the semi-apochromat, and explained how perfect its performance is when well made and used with a suitable screen to cut off the secondary spectrum, but for the critical work of the advanced student, or for the minute investigation of the philosophical inquirer, the more expensive rival must take the precedence. Seeing apochromats were originated by the genius of the late Professor Abbe, and manufactured first by the justly eminent firm of Carl Zeiss, it is only to be expected that the productions of this firm should be, as

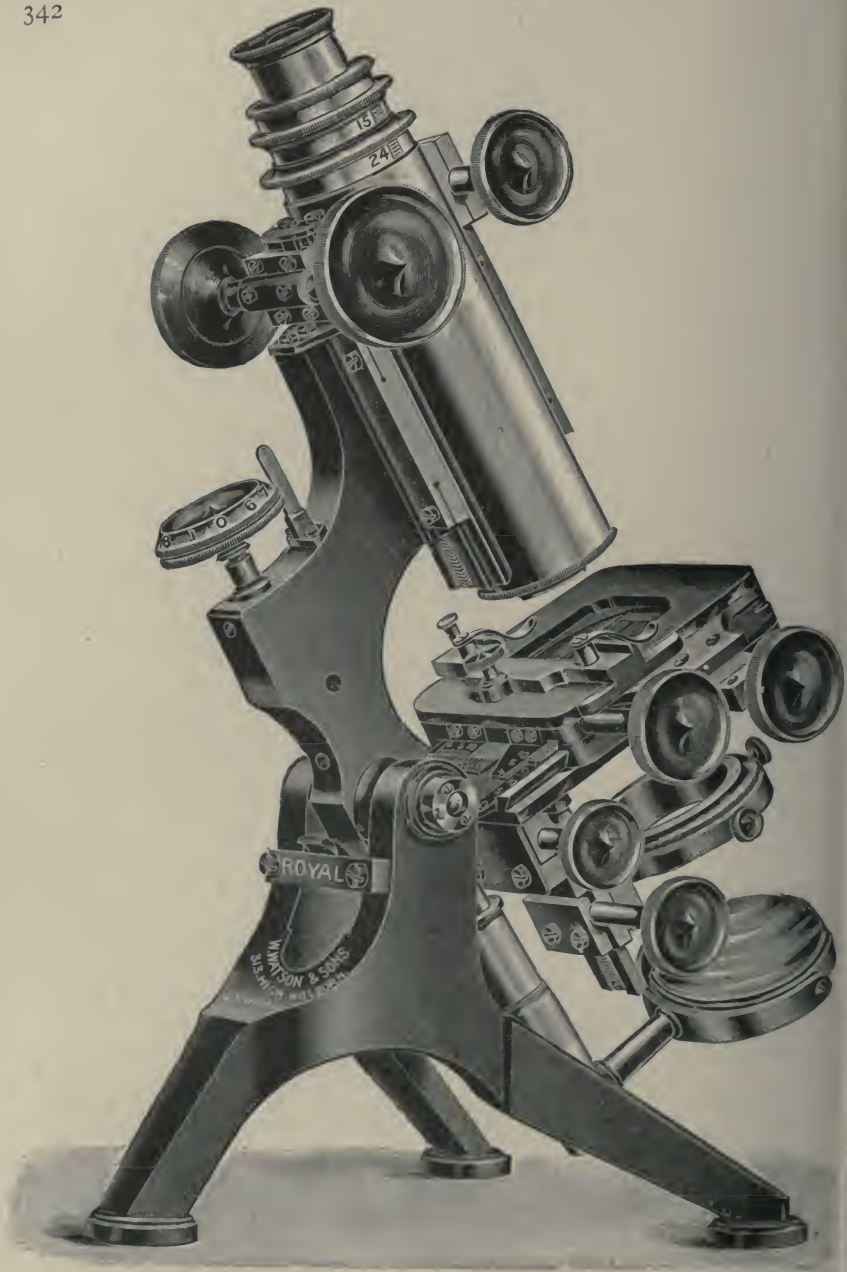


Fig. 204.—Watson & Sons' "Royal" Microscope.

indeed they truly are, of the highest order of merit. The excellence of definition of these objectives coupled with the absence of practically all colour when dealing with specimens of all sorts, renders these combinations of the very greatest possible value for final examinations and critical work of all sorts, whether it be of objects requiring low-power magnifications or those that demand the greatest resolution and amplification possible. The 3-mm. and 2-mm. combinations are especially superb in their performance, and, were it not for their necessarily high price, would be in more common use. Their outer zones being as near perfection as it seems possible for the mathematician to compute and the optician to construct with our present knowledge, so their performance with oblique light leaves but little, if anything, to be desired, although we trust a time will come when with direct light they can be used with a full cone as well as they can be now with a $\frac{3}{4}$ one.

A reputation of this description, so justly held by this firm for now so many years, has necessarily led others to make very strenuous efforts to compute objectives of the same merit. Indeed Messrs. Powell & Lealand constructed, shortly after the advent of the apochromat, a competing lens—a wonderful one, we have been told, at the time, but which the same firm has long since replaced by others more perfect. One of these latter constructions we used with much profit wherewith to take a very large proportion of the photomicrographs in the *Atlas of Bacteriology* published by the Scientific Press some years ago, being especially designed to give *a large* flat field.

But Continental manufacturers—of late especially—have been exceedingly hard at work raising the tone and quality of their apochromats until at length they have reached a goal of perfection that defies description. To these we feel bound in common fairness at once to refer.

Signor Koristka of Milan produces apochromats which—after a very extended and severely conducted series of experiments both with the test-plate and with test-objects—are all of *the very highest possible order of excellence*. We have seen, through his courtesy, all his manufactures, and all are equally good. The 1·5-mm, a *spécialité*, with a very long working-distance for so high a power, is an exceedingly fine lens and produces an image so absolutely perfect as to render any distinction between

it and that displayed by two apochromatic twelfths—one for the short and one for the long tube—by Zeiss impossible. Comparison with Swift's new apochromatic twelfth should also be made.

HERR LEITZ OF WETZLAR, too, has given much attention in the same direction, and produced magnificent specimens of workmanship that are beyond praise. His 2-mm. is one of the finest combinations we have met with both when using the test-plate as well as test-objects ; and the rendering of the dots in an excellent specimen of Amphipleura pellucida mounted in realgar by Dr. van Heurck leaves absolutely nothing that can be desired by the most critical observer.

HERR REICHERT OF VIENNA has not been neglectful in bringing up his apochromats to the level of excellence attained by others of which we have justly and truly spoken so highly. His 2-mm. is one of the most remarkable combinations that have ever passed through our hands. Whilst showing the tertiary spectrum with the plate—an explanation of which is spoken about at some length in the article devoted to the testing of lenses—its performance as a combination for producing resolution of the finest details of the minutest structure *has no superior in the world*, the image being so crisp and beautiful. It is a very remarkable lens. His 4-mm. apochromatic objective is one, too, that competes with those of similar focal length and aperture by Zeiss and Koristka, and a new combination by Watson.

Speaking of the 2-mm. objective as made by these four manufacturers, Koristka, Leitz, Reichert, and Zeiss, the manner in which they show the dots in Amphipleura pellucida even with green light and a powerful electric lamp, must be seen to be fully appreciated ; the reproduction in Fig. 1, Plate VI., of a photograph taken with the Zeiss 2-mm. apochromatic and blue illumination furnishing but some idea of the images of which we speak. This photograph was awarded (with some others) a Gold Medal at the St. Louis Exhibition, 1903, and we have often felt that the honour should have been placed in the manufacturer's hands rather than in those of the simple photomicrographer, seeing it is not so difficult to take a photograph—although at this great amplification it is not quite an easy matter—as to compute and construct a lens that will plainly show dots not much more than $\frac{1}{100000}$th of an inch in diameter in the wonderful manner exhibited. We have also taken photographs of the same diatom

with 2-mm. objectives by Leitz, Reichert, and Koristka, of gradually diminishing amplifications so as to show the reader the appearance presented with oculars of smaller and smaller magnifying power (Plate XIII., Figs. 5 and 6, and Plate XV., Fig. 2). When the optician's art rises to this pitch of excellence, and a difference is required to be shown between objectives where possibly no distinction exists, and a selection to be made where all appear to be of equal merit, the task of the expert becomes simply an impossible one, and the only thing open to him is to congratulate each manufacturer and acknowledge his inability.[1]

So many are the varied uses to which the microscope can be employed, that the reader can readily understand the difficulty we should experience if we attempted, alone and unassisted, to state very positively the objectives most suitable for every particular line of investigation. We have felt this so strongly that, fearing we might unintentionally lead the student astray by our attempting to do so, we have written to several microscopists whose experience and work upon special subjects justify their speaking with the voice of authority. Doubtless we have not made an exhaustive list of inquiries, hence we should like to add, if we have failed to apply to any specialist upon any given subject, whose aid we ought to have sought, he must regard it not as a sin of *com*mission, but merely as one of *o*mission on our part, that has simply arisen from an unfortunate oversight. We met with a hearty response, and the substance of the replies we now append ; taking this opportunity of thanking the writers for allowing us to lay before our readers their individual experiences, which we know will be highly appreciated.

[1] In mentioning these four opticians as producing such remarkably fine objectives we feel that we may be doing an injustice not to include the work of Dr. Hartnack of Potsdam—the representative of the ancient firm of that illustrious name. He had, however, but commenced his apochromatic series, and formerly only listed a twelfth and fifteenth. The twelfth we like the better, and is certainly a very fine combination, although we admit it exhibits more colour than we are accustomed to see with the Abbe Plate. Notwithstanding this it performs admirably with Amphipleura, showing the dots very well, and conforms very satisfactorily with all the other tests given later in this book. It is sold in a most convenient *form* of mount, but requires an adapter to fit it on to the modern microscope, which we think a mistake, seeing the universal fitting is now so generally adopted.

Mr. F. P. SMITH, formerly Hon. Editor to the Quekett Microscopical Club, wrote: "I usually use a 2-in., 1-in., and ⅔rds for ordinary spider work, employing a ¼-in. for detailed examinations. By far the best way to examine a spider is to put it into a pomade-pot lid, cover it with methylated spirit, and treat it as an opaque object, using of course low powers. If suitable for preservation it can be subsequently mounted and used as a transparency, when I employ the ¼-in."

Mr. CHARLES D. SOAR with the Hydrachnidæ used a 1½-in. and ½-in., and sometimes a ⅙th, employing dark-ground illumination whenever possible. The 1½-in. served to identify species and the ½-in. supplied details especially for "drawing" purposes. The ⅙th, however, was more or less exclusively employed for the examination of skin texture.

Mr. W. WESCHÉ only had three objectives in constant use for his well-known anatomical researches. An inch he employed upon the Binocular Microscope, a ⅔rds as a finder, and a ⅛th, with as great a working-distance as possible, for details of structure.

Mr. A. E. HILTON—concerning the study of Mycetozoa—wrote: "For sporangia I use a 2-in. and a 1-in., and mostly these are sufficient for my purpose, as great magnifications for my class of work in general are not required. When, however, the Capillitia and spores have to be studied, I employ a dry ⅛th, although I should recommend in preference an immersion $\frac{1}{12}$th, and I gather Mr. Massee is of the same opinion, for occasionally we require a magnification of 1200 diameters."

Mr. RICHARD LEWIS, who from his long experience was a distinctive authority, used to say he had a set of objectives ranging from 3 in. to ⅙ in., all of which he found of frequent use in the study of Ixodidæ and the sense organs of insects. For the majority of Ticks the 2-in. and 1-in. were sufficient if employed on a binocular stand, but when details had to be searched for a ½-in. was more useful. This, he added, may not always be of sufficient power, and if expense was of no object the higher power apochromats will be found most serviceable.

Mr. JAMES BURTON, for work on Algæ and Fungi, employed low powers, but occasionally a ¼th and a ⅓th and rarely an ⅛th. He preferred all objectives with low numerical aperture so that very great depth of focus was obtained.

Mr. H. E FREEMAN, who was interested in and has studied at some length the Acarina, with the life history in general of microscopic insects, employed a 3-in., 1½-in., and ⅔rds with opaque objects in small sunk cells. He objected to the use of very high powers because of the difficulty in following a moving object about the field of view.

Mr. T. R. ROSSETER, for his well-known researches upon Entozoa, especially, perhaps, the avian Cestoda, used to employ a 2-in. and ½-in. of *second-class type* for rough use, and an inch, ½-in., ¼-in., and ⅛th of high-class make, with a $\frac{1}{16}$th water immersion occasionally to study the details when in search of minutiæ.

Mr. HERBERT S. MARTIN, when working at his favourite study of Petrology, found an inch and a 2-in. of sufficiently high power for ordinary examinations of rocks, reserving a ⅙th for "viewing the interference figures formed at the back of the objective when convergent polarised light is used."

Mr. ALBERT ASHE (whose experience was somewhat unique, his studies having been directed more especially to animal, vegetable, and mineral substances for industrial purposes, such as adulterations) rarely employed other objectives than a second-class ⅔rds and a ⅙th, quite a plain form of stand being sufficient. But for delicate work arising occasionally, he invariably employed apochromatics, presumably of the same focal length with a suitable condenser and critical illumination upon a first-class stand.

Mr. E. LEONARD, who has given so much attention to the mounting of diatoms and their selection, said his battery consisted of a 1¼, ¾, ½, ⅙, and $\frac{1}{10}$th oil immersion. For mounting, the two lowest powers were employed, the 1¼ for searching over the "drop" on the slide, the ¾ for the actual mounting. Occasionally the ½-in. had to be used for the "drop" searching, and of course, for subsequent work of a more delicate nature, the $\frac{1}{10}$th. For "picking up" the best objective was a 1¼-in., and for dark-ground illumination a ¾-in. He added, "the best bristles for mounting with I obtain from the back of my fox-terrier!"

Mr. ARTHUR EARLAND, for some years the valued Hon. Sec. to the Quekett Club, whose extensive work upon Foraminifera and Radiolaria is too well known to need any remark, said he rarely employed any other objective save a 1½-in., a ⅔rds, and a ¼-in.

Mr. C. F. ROUSSELET, one of our greatest authorities upon Rotifera, preferred for his work the 36-mm. (not to be confused with the 35-mm.) and the 17-mm. for the binocular, the apochromatic 12-mm. for the long-tube monocular instrument, and the 2·5-mm. Zeiss water immersion apochromatic for studying the fine anatomical structure of *living* specimens in water.

Mr. G. C. KAROP, M.R.C.S., L.S.A., formerly so very many years the valued and highly esteemed Secretary to the Quekett Club, an amateur who has devoted his attention, it may be said, to almost every variety of subject, and whose experience is consequently almost unique, wrote: "If I were asked the two most useful objectives for an amateur to purchase they would be a $\frac{2}{3}$rds and a $\frac{1}{6}$th. If I had to select three, I should add a $\frac{1}{12}$th, and if yet one more, it would be a $1\frac{1}{2}$-in."

Mr. H. MORLAND, in his work upon the Diatomaceæ, more especially relating perhaps to their classification and mounting, wrote he rarely found he required objectives other than a quarter-inch as a high-power, and, say, an inch for other purposes. He did not make so especial a study of the secondary markings, and so had no need for very high-power immersion objectives.

Mr. A. STILL found for his work upon Microalgæ and Desmids an inch, $\frac{2}{3}$rds, and a $\frac{1}{6}$th all he required, the low-powers being more especially for searching and the $\frac{1}{6}$th for examining details.

Mr. G. MASSEE, past-President of the Quekett Microscopical Club (four years), wrote: "The objectives I find most useful for work on Fungi and Myxomycetes are:

"(1) For a general aspect of the superficial structure a $1\frac{1}{2}$-in.

"(2) For histological details, measurements of spores, a $\frac{1}{6}$th, as by common consent a magnification of 400 diameters is used.

"(3) In the systematic study of the Myxomycetes, and in some special groups of fungi where the so-called species run very close and the finest details are required, I use a $\frac{1}{12}$th."

Mr. DAVID BRYCE found in his work upon Rotifera he mostly used two objectives, a 1-in. and a $\frac{1}{4}$-in: the former for searching through specimens and the latter for examining details. He added: "The student should recollect my subjects are alive, and average about $\frac{1}{50}$th of an inch extreme length, so that every movement takes them out of focus," which, he subsequently pointed out, prevents the employment of higher power objectives. Occasionally, however, he had required a $\frac{1}{6}$th.

Mr. ROBERT PAULSON, speaking of the work at The Laboratory, London County Council Technical Institute, Lalham Crescent, W., wrote he had usually found a $\frac{3}{4}$-in. and a dry $\frac{1}{8}$th all he required for his work in biological study, but occasionally, however, he had employed a $\frac{1}{12}$th.

Mr. ARTHUR COTTAM, F.R.A.S., whose opinion always commanded immediate respect, used to say he employed a Zeiss A (15-mm.) objective for selecting and mounting his diatoms, as its shape is peculiarly convenient, but that with very small specimens he employed all types of the highest powers suitable to the difficulties of the situation.

Mr. HOLDER, who pays so much attention to biology, and is such a successful microtomist, seemed to use exclusively an inch and a $\frac{1}{6}$th for all work in general.

Mr. SIDWELL, whose work upon the Entomostraca is so well known to microscopists, wrote to the effect that as regards the best objectives for pond life—for the examination of Entomostraca—he found an inch N.A. ·28 on the binocular the most useful, but employed a half-inch apochromatic for objects possessing smaller details. Occasionally, however, it was necessary to use a higher power when he resorted to a $\frac{1}{12}$th immersion.

With respect to the study of the Diatomaceæ, the author of this work is bound to admit the use of all types of objectives ranging from the lowest to the highest powers. A $\frac{1}{6}$th apochromat is a very excellent one to employ upon diatoms for primarily studying their forms, but their *secondary markings* require the finest apochromatic 2-mm. or 1·5-mm. with the aid of green or (better still) blue illumination.[1] The quarter-inch apochromatic by Zeiss on the long tube is a much neglected objective, and is one especially convenient, having a longer working-distance (some specimens mounted years ago having rather thick covers) than a $\frac{1}{6}$th. So, too, for the same reason the 3-mm. apochromatic may often be more useful than the 2-mm. The third or half-inch for sorting specimens are both of service; and the 1-in. apochromatic (used with a high-power ocular) shows the colours of diatoms remarkably well.

[1] For the new blue screens, see Addenda.

CHAPTER XIV

TESTING OBJECTIVES

THE testing of objectives has always been an interesting though difficult subject. It used to consist in examining the performance of the lens upon certain specimens called test-objects, but nowadays, besides this method, there is one of a far more searching character originated by the late Professor Abbe, called after him "The Abbe Test-plate," a description of which we at once proceed to furnish.

The Abbe Test-plate

In the construction of this excellent and useful little piece of apparatus the special aim of its inventor, Professor Abbe, was to artificially produce an object as full of pronounced contrasts as possible. For this purpose advantage was taken of the densely opaque character of a chemically deposited layer of pure silver on a piece of glass, for it is evident if a film of the nature described be ruled in such a manner as to remove the deposit and leave clear glass in certain lines—the film being left untouched between—the result would appear under the microscope as a kind of coarse grating. Now, an object consisting of an alternating series of dark and bright lines fulfils the very ideal required, for it is difficult to imagine one that could be constructed having greater contrasts.

The deposit on the Abbe plate is not always absolutely black although exceedingly *dense*, and may then be described as consisting of a dull grey semi-opaque "filling" transmitting but very little light indeed, in which are closely scattered numerous black irregular-shaped particles of silver deposit. Although these are closely packed together in the filling, still they appear sufficiently discrete to offer every facility for accurate focussing.

This is the kind of plate we prefer, but sometimes the so-called black lines are black indeed, in which case the particles are indistinguishable, and only the actual edges of the lines are capable of being focussed.

With a superficial examination, the test-plate appears to consist simply of an ordinary 3 × 1 in. glass slip, having cemented upon its upper surface six small circular silvery-looking cover-glasses (Figs. 4A and 4B, Plate I.) : but on closer inspection each one exhibits, even to the naked eye, a faint indication—plainly seen with a hand magnifier—of the minute rulings to which reference has been made.

All the cover-glasses are of specified thicknesses varying from ·09 to ·24 mm., the exact measurements of each being etched in white figures beneath it on the slip. Upon looking at any one of these six covers—preferably the one marked ·18—with, say, a $\frac{1}{12}$-in. semi-apochromatic, it will usually be found to present four batches of lines, each batch consisting of eleven white-spaced rulings, called technically " the white lines," between twelve black or opaque ones. The white spaces and the black lines are not of quite equal width, the former being the little narrower of the two in most instances that we have met with, although such is not always the case.

Further it will be seen whilst using *direct* light, that when the black lines are out of focus, the white ones immediately appear to have coloured fringes at their upper and lower borders and that the tint of these fringes varies according to whether the objective be pushed within the focus, or pulled without it. If the former, the upper and lower borders in question show purple fringes (appearing as if actually coming from the black lines adjacent) ; whilst if the latter position has been taken for the objective, the purple colour has vanished and apple-green has taken its place. Putting this in short, the colours exhibited are purple within the focus and apple-green outside it. It will also be noticed by the careful observer that whether purple or apple-green be in evidence, both sides of the white lines are similarly coloured. This is technically called " symmetrical colouring of the lines."

Should, however, direct light be now changed into *oblique* light—and here it is necessary for accurate explanation that such shall be produced by placing a card over the lower two-thirds or three-fourths of the condenser between it and the light, so that

illumination can only *enter* through its uppermost third or fourth (appearing as the *lowest* third or fourth of the back lens of the objective as seen when looking down the tube of the microscope)—the arrangement of the colour effects becomes entirely different.[1]

First. The colours at the upper and lower borders of the white lines respectively are now no longer symmetrical, for with a visually corrected semi-apochromatic objective of the best quality, apple-green usually appears at the upper border, whilst purple is seen at the lower one of each of the white spaces.

Secondly, it will be found that no alteration of the objective, that is to say whether it is placed within or without the focus, causes any change of colour effect as it did when using direct light; and—

Thirdly, that no alteration of colour can be produced by any means whatever excepting by admitting light from the exactly opposite third or fourth of the substage condenser to that employed as we have just stated.

It will be of interest before proceeding further, and to make what follows better understood, if the cause or causes of these colour effects be carefully explained. For that purpose, before actually commencing to do so, the reader is referred to Figs. 69, 70, 71, and 72 in the chapter upon the improvements effected in the construction of modern objectives. In this chapter it has been explained achromatism consists in a folding over of the spectrum at the particular colour selected by the optician for the best correction, and that the rays of this "preferred colour" then have the shortest focal length, the others meeting at intervals along the axis forming the colours of the *secondary spectrum*.[2] The point then here to bear in mind is that the apple-green *has the shortest focal length and the purple*

[1] This can be done with the Abbe illuminating apparatus by simply closing the iris and by turning the milled head which puts the iris out of axial centrality.

[2] The so-called primary colours are those which come into evidence when a prism or grating breaks a beam of white light into its components—rainbow colours, as they are often called—consisting of red, orange, yellow, green, blue, violet, and ultra-violet; but the secondary colours are those formed by unions of these different residuals, such as apple-green being formed by the joining of yellow and green, and purple by the union of red and violet.

the longest, the intermediate mixture of orange and blue being omitted for clearness of description. Let Fig. 205 be now considered. We have here a point of light P situated upon the axis of a semi-apochromatic combination roughly shown at L. In an exaggerated manner the yellow-green rays—because they have the shortest focal length in the construction selected—are seen focussing at A, whilst the red and violet forming the purple, having the longest focal length, meet at A′. If now a section of the beam of light were possible at A, we should see an apple-green *point of light* surrounded by a faint haze of purple; whilst if we examined a section at A′ we should see a purple point

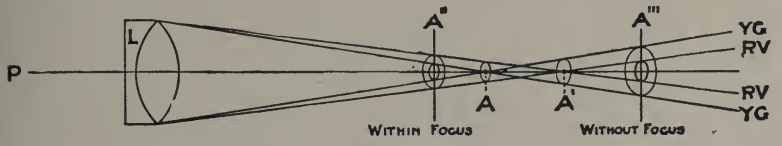


Fig. 205.

surrounded by an apple-green halo (the rays forming this halo being shown to have crossed after forming a focus at A).

Within the focus at a plane marked A″ we should see an apple-green *disc* of light surrounded by a distinct and well-marked halo of purple, whilst considerably without the focus at A‴ a purple *disc* surrounded by a halo of apple-green.

Although in the Abbe test-plate a white line of sensible area between two black ones is the object, instead of a discrete point of light, a little thought will suffice to show that the same reasoning affords an equally correct explanation of the colour phenomena in this instance, as in the previous one. Perhaps the easiest way to understand this is to remember in the first case the image of the point was formed in the yellow-green, hence in the Abbe plate the image of the white line is really in apple-green also.

Now when the objective was lowered in the first experiment the image of the point became blurred into an apple-green disc with a halo or fringe of purple around it; so in the second, the image of the white line following the same course becomes blurred and the purple fringe makes its appearance at the edges of it. Likewise also, when the objective was raised in the first

instance outside the focus, the purple point became blurred into a disc and had an apple-green halo around it; so in the second, the image of the white line becomes blurred and the apple-green fringe forms above and below it.

When employing oblique light formed in the manner already described, the conditions were materially changed.

Fig. 206 is drawn in exaggeration to explain the situation. The rays from the edges of the white line AA are shown as entering and leaving *the back lens* of an objective at its lower third, as would be seen in fact if the observer were looking down the tube of the microscope, the ocular having been removed.

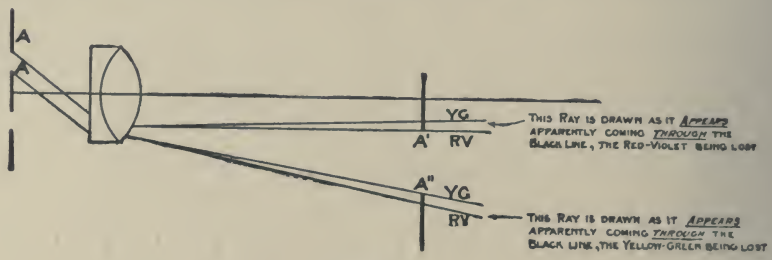

A
A
YG
A' RV
THIS RAY IS DRAWN AS IT APPEARS APPARENTLY COMING THROUGH THE BLACK LINE, THE RED-VIOLET BEING LOST
A"
YG
RV
THIS RAY IS DRAWN AS IT APPEARS APPARENTLY COMING THROUGH THE BLACK LINE, THE YELLOW-GREEN BEING LOST

Fig. 206.

The *image* of the white line AA is shown diagrammatically at A′A″. Here at A′, the upper edge, the yellow-green colour appears because it is the *most* refracted of any of the colours of the spectrum, and, having the shortest focal length, so lies at the very edge A′: whilst at A″ we see the purple tint because that colour, being the *least* refracted (longest focal length) consequently lies at the lowest limit of the image. The red-violet ray is lost in the former case and the apple-green in the latter because of the brilliancy of the white line.[1]

That in this case no change of position of the objective causes any alteration in the colour fringes is accounted for by the fact that no movement of the microscope up or down alters the condition of things in the slightest manner, hence the absence of any change of colour.

The exact reversal of colour effect produced by changing the card beneath the condenser, and so allowing the light to enter

[1] With practice, however, these colours *can be distinctly though faintly seen*, notwithstanding the general brilliancy of the white line.

from the opposite side, is readily understood, because the position of the least refracted ray becomes that of the most, and *vice versâ*. This concludes an explanation, in general, how the tinted fringes are formed both with direct and oblique light in the Abbe test-plate.

The next point which strikes the observer, even if it has not been noticed before in this cursory examination, is that the whole field of view has never been in focus at one and the same time. It is not so even if a lower power ocular than the × 12, which is supposed to have been used throughout these experiments, be tried. Indeed it would seem as if nothing more than about the central and intermediate zones of the field can be focussed sharply simultaneously.

We believe that, so far as at present is known, no hope can be held out for anything better. It is this inability to produce a flat uniformly defined field at one and the same moment, that has led several microscopists of the old school to say that they consider, in their opinion, the modern objective is retrograde, adding that they do not hold much improvement has been effected by the optician in recent years, seeing that the field of perfect definition is so much smaller than in some of the old objectives with which they are acquainted. To this it must be replied that at all times, whether now or in the past, the equalisation of the definition in all three zones of the field of view is the effect of a compromise. If the best definition is required in the central and intermediate zones (occupied alone by most objects of microscopical interest), then the outer one must be left to suffer, with the possibility of being immediately brought into focus by a touch of the fine-adjustment screw; whereas, if the whole field be required to be equally well defined, it *must and can only be produced* by lowering the quality of the definition of the central and intermediate zones. In the present day the former state of things being preferred by most microscopists is the cause for the apparent falling off of the performance exhibited by this outer zone so noticeable in modern lenses. It is needless to remark that the three zones of *the field* must not be confused with the three zones of the *back lens of the objective*, for they have no relation one with the other, otherwise cutting off the outer zone of the back lens by the iris would cause a contraction of the field of view, which it does not.

Precautions to be Observed before Commencing to Test the Performance of any Objective with the Abbe Test-plate

1. The draw-tube must be set the exact distance for which the objective is corrected: it is frequently engraved upon the side of the mount.[1]

2. The back lens of the objective must be filled with light; hence every objective over N.A. 1·0 must be used with an immersion condenser oiled to the slip and of approximately equal N.A., to make the observation of scientific value. The condenser should be carefully centred.

3. The cover-glass of the test-plate to be selected should be ·18 mm. or thereabouts, because that is the average thickness selected by most opticians.

4. The compensating ocular is to be employed for all apochromatics, no matter what their focal length, and with semi-apochromats of over, say, ·5 N.A.; but the ordinary achromat and the low-power semi-apochromats below the N.A. mentioned perform best in most cases, although perhaps not in all instances, with the ordinary Huyghenian ocular.

5. The magnifying power of the ocular is to be such that multiplied by the initial of the objective, the total amplification should numerically equal the N.A. × 1000; hence a $\frac{1}{12}$th of, say, 1·4 N.A. requires a × 12 ocular.

6. The illumination must be critical and very brilliantly *white* so far as possible. There must be *no obliquity* in the direction of the light from off the mirror, by which is meant the beams from the illuminant should pass *centrally* through the condenser.

Testing Objectives

Examination of Semi-apochromatics: $\frac{1}{12}$-in. or $\frac{1}{18}$-in. N.A. 1·3 to 1·4

A. With the Abbe Test-plate.
B. With Special Test-objects.

A. **With the Abbe Test-plate.**—The list of precautions given in the preceding section having been duly read and observed, the examination of an objective N.A. 1·3 or 1·4, say a $\frac{1}{18}$th,

[1] A qualification of this statement is made later.

$\frac{1}{12}$th, or $\frac{1}{8}$th, is carried out in the following manner for two purposes: to ascertain the correction—

(*a*) For *chromatic* aberration (testing for colour).
  (i) The appearances presented with direct light.
  (ii) „ „ „ oblique light.
(*b*) For *spherical* aberration and definition.
  (i) With *direct* light.
  (ii) With *oblique* light.

(*a*) (i) When the *edges of the black lines are in accurate* focus, care being taken that a *white* space occupies as nearly as possible the true diameter of the field; *no colour* phenomena should be visible. If much be present, it may be due to the compensation of the ocular not quite suiting the individual objective under observation. Let the former be removed and replaced by a Holos[1] compensating eyepiece of similar magnifying power. If the ocular has been at fault, adjustment of the component lenses of this ocular will be found to correct it. If, however, no improvement takes place, this appearance does not betoken a good objective; *but it must be borne in mind the accurate focussing of the edges of the black lines* bordering the white space must be carefully sought after, for it is only at the very moment of their accurate focus with the fine adjustment that they should appear colourless.[2] The instant the objective is lowered beneath the focus or raised above it, colour *should* appear.[3] What colours are to be expected depends upon the position

[1] These are sold by Messrs. Watson & Sons, the improved series being an advance upon the original.

[2] Another word of caution might be mentioned. We have spoken of the necessity of having critical light; but it is important also to see that the mirror does not reflect the beams of the lamp with *any degree of obliquity at all* upon the condenser. By this we mean that if, for example, a point of light from the illuminant were in use, the microscopist should see that that point is in the centre of the field of view when the plate is in use with direct light. Should any colour (otherwise than the folding-over one) be seen then *upon focussing the lines* (which we have explained should not be the case), it is possible for it to be caused by the mirror being a little obliquely placed; hence, before coming to any conclusion on the matter, the simple experiment of shifting the mirror a trifle should always be made, to see if that does not at once remedy the fault in question.

[3] Too great stress cannot be laid upon the fact that with a first-class objective the slightest touch of the fine adjustment puts the object out of focus.

chosen by the computer of the system for the folding over of the spectrum; in other words, which colour shall have the shortest focal length, and consequently in what colour the image shall be formed.[1] If he select what is often called the apple-green correction, then such folding over usually takes place about wave-length 5500 in the ordinary spectrum, and the colours exhibited on the borders of the white lines, symmetrically placed, are purple within and apple-green without the focus. This class of correction is a great favourite, but a few makers pitch their folding-over point slightly nearer the green, which produces a redder purple within the focus and a deeper green outside it. This is called a slightly *under*-corrected combination, a term to be explained a little later on.

Sometimes the colour outside the focus is a very light yellow-green indeed, and quite a blue-purple within it. This type is called a slightly *over*-corrected objective, a term also needing explanation hereafter.

Lastly, an objective might present (although they must be very rare and few in number in the case of $\frac{1}{12}$th or $\frac{1}{8}$th, for we have never yet met with one) the white lines bordered with blue *outside* the focus, but a red-orange within it. This denotes a correction for photography only, and would be a wretched objective for visual purposes.

(*a*) (ii) The objective has now to be tested with oblique light, and it is important that this should be obtained in the following manner:—The condenser must be covered up by a card throughout its lower two-thirds or three-fourths, preferably one with a curved edge—crescent-moon shape—so that when looking at the back lens of the objective down the tube with the ocular removed for the moment, light only appears to enter by a little portion at its *lowest* limit.

On returning the ocular and looking at the lines, care should be exercised that one of the white spaces should occupy as

[1] The student must recollect that the image of the object *must* be coloured when using a semi-apochromat, and that such colouring is in accordance with the wave-length of the ray having the shortest focal length. It is only the apochromat that can furnish a really pure white image. If the performance of the two different kinds of objectives be closely compared when a diatom is the object, the truth of this remark is experimentally put to the test. The apple-green colour, however, gives an image not easily distinguished from white unless the comparison as described above be made at the moment.

nearly as possible the true diameter of the field; that is, centrally from side to side.

Three white lines are usually capable of being seen with the × 12 ocular and the $\frac{1}{12}$th objective; but occasionally the screening diaphragm of the eyepiece may cut off a portion of the white spaces at the upper and lower parts of the field.

The fringes on the upper and lower borders of the central white line will now be found to be of *different colour*, the upper being of apple-green and the lower of purple; that is, of course, presuming the objective to be the best visual type. There should be no fluffiness, and the edges of the lines should be capable of being sharply focussed.

It is possible by this time that the student may have noticed the colours in the upper white space may not be quite the same as those in the central, and again those in the central may not be quite the same as those in the lowest. This arises from the eyepiece not being quite in compensation with the objective. Indeed, it may be said to be almost an impossibility to prevent a *little* difference; but if it is very pronounced the ocular is probably the cause of trouble and not the objective. This may be proved by altering the adjustment of a Holos eyepiece, when it will be found it can nearly always be greatly modified.[1] To remedy this disturbing effect with the ordinary compensating ocular whilst testing an objective, we have already said, the *border* of the central white space *under observation* at the moment should be placed in the *exact diameter* of the field and then the colour carefully noted, the test-plate being shifted so as to put the other border in the same position before noting its colour. By this means any change of colour due to the eyepiece is eradicated.

To test independently the three zones of the objective for colour correction it is almost imperative for the microscope to have an Abbe substage illuminating arrangement, as the iris diaphragm when shut somewhat closely—in fact, down to the size of a large pinhole—has to be shifted from centrality across the field from above downwards to the periphery of the lens,

[1] The high-power Holos ocular in the original series had a very inconveniently near eye-point, so much so that many microscopists found it exceedingly difficult to use in general work. In the new series, however, this defect has been overcome to a very great degree.

whilst the eye is placed at the ocular, noting any changes of colour during the operation. The precaution above explained, to eliminate any error in the compensation of the ocular by keeping the colour under observation in the exact diameter of the field, should be rigidly carried out. It will be usually found that when the little aperture of the iris is placed in the central zone, the lines are dull and ill-defined. This is caused by the bright reflecting surface of the *upper* face of the silver. When the lighting is perfectly straight, some of the rays passing through the slits (or white spaces) are reflected from the *inner* surface of the front lens *back again* upon the cover-glass, the silvered portion of which turns them back again into the objective, and thus *fogs* the dark lines.

As the small illuminated area (caused by the pinhole iris) traverses the intermediate and especially the outer zone of the back lens of the objective, the colours of each border of the white line may slightly alter in some lenses, but in the very finest it remains nearly—if not actually—constant. This means that in many lenses the outer zone is not corrected quite so accurately as the rest : in point of fact it has appeared to the writer this zone is often slightly *under*-corrected.[1] The upper border of the white line with oblique light, we have already stated, shows the point of folding over of the spectrum, and thus indicates whether the objective be over or under-corrected; but when using this exceedingly small point of light, the illumination is always so faint that it becomes very difficult to say whether the upper border is fringed with apple-green, a lighter yellow-green, a deeper green, or very dark green ; hence it will be found practically to be of more utility for a beginner, in *this* case, to be guided by the colour presented by the *lower* border of the white line instead—it being placed of course in the true diameter of the field. If this appears of a purple colour throughout the movements of the pinhole iris, the three zones are perfectly corrected ; if the purple becomes redder and redder, perhaps a distinct red, then the objective shows a slight *under*-correction; whereas if it change to violet instead—or blue—then *over*-correction is present.

Sometimes the colours are violently different in the central intermediate and outer zones; this is bad, for in a really fine

[1] These terms of *under* and *over*-correction will be explained very shortly.

objective, as before stated, there should be but little if any change—anyhow, not of that violent nature.

Seeing the terms "under" and "over-corrected" have not as yet been explained, it may be well at once, before proceeding further, to do so.

It has been mentioned in the visually constructed objective that the position mostly chosen for the folding over of the spectrum is in the apple-green, say about the wave-length 5500 in the normal spectrum; but if now the folding over takes place nearer the violet end, it is called *under*-correction, whilst if towards the red end, *over*-correction. To make what follows more easily understood, it is a convenient method to take a slip of paper some 8 in. in length, and to draw a line along it for 6 in., marking off seven dots at a space of 1 in. apart, as shown half size in Fig. 207.

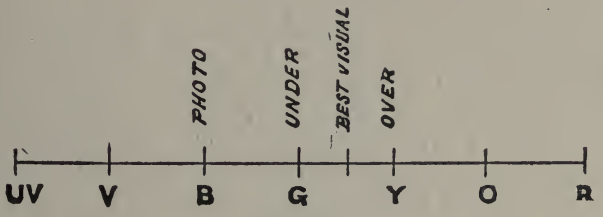


Fig. 207.

Commencing at the right-hand end of the line, let the following letters—R, O, Y, G, B, V, and UV—be affixed to the dots in succession. Midway between Y and G, at right angles to this line, let another be made, marking it with the words "Best Visual." A bending over of the slip of paper at this achromatising line plainly illustrates how Fig. 71 was obtained in theory, for it immediately indicates by mere inspection how the folding together of the different colours has caused the blending about which mention has been made; in other words, it reveals the formation of the secondary spectrum.

Let, now, three additional lines be drawn at right angles to the long horizontal one, the point at G being marked "slight *under*-correction," the second at Y "slight *over*-correction," and the third at B, "*photo* correction."

When a folding over of the slip is made at G—as occurs in

slight *under*-correction—a union of yellow and blue and of orange and violet will follow, whilst it will be observed red and ultra-violet are left outstanding. The latter colour (UV) is so feebly perceived by the human eye that it may be left out of consideration for our present purpose. Hence when the white line in the Abbe plate illuminated by oblique light (in the manner prescribed) is looked at with an *under*-corrected objective, its *lower* border will have a reddish fringe, whilst its *upper* one will have a pronounced green tint.

With an *over*-corrected lens, where the folding over takes place in the yellow, the slip of paper will now show, if bent at that position, a union of orange with green, and of red with blue, leaving violet outstanding; hence the *lower* margin of the white line will now exhibit a colour more nearly violet than purple, and its upper margin a fringe composed of almost a pure yellow.

With a *photographic correction* (rarely to be met with in high powers, or indeed any over, say, a half-inch), where B is the folding-over point, the slip will indicate a union of violet with green, whilst yellow, orange, and red are left outstanding. Hence the upper border of the white line shows blue, and the lower one a mixture of the three colours yellow, orange, and red.

Arranged in the form of a table for convenience of reference, these different colour-effects become as follows:

Exhibition of Colours at Upper and Lower Borders of the Central White Line with Oblique Light obtained in the Special Manner described.

—	Point of folding over.	Upper border shows—	Lower border shows—
Visual correction of what is mostly called the highest order.	Apple-green.	Apple-green.	Purple.
Slightly under-corrected combination.	Green.	Green.	Reddish purple.
Slightly over-corrected combination.	Yellow or *very* yellow-green.	Yellow or *very* yellow-green.	*Very* blue-purple or violet.
A purely photographic correction.	Blue.	Blue.	Orange-red.

It is evident from this table, and from what has been said, that, when using oblique light in the manner described, the tints exhibited respectively by the *upper* and *lower borders* of the centrally placed white line afford most valuable information to the examiner of the objective.

The colour exhibited by the *upper border* shows three things at one and the same time:

(1) The colour in which *the* image is formed.
(2) The colour having the *shortest* focal length.
(3) The point of folding over of the spectrum.

Moreover, if this colour be *yellow*, it shows *over*-correction.
" " " *green*, it shows *under*-correction.
" " " *apple-green*, it shows *usual visual* correction.
" " " *blue*, it shows *a purely photographic* correction.

The colour exhibited by the *lower* border, if it be—

Blue-purple or blue shows *over*-correction.
Reddish purple to red " *under*-correction.
Purple . . . " *proper visual correction.*
Orange-red . . " *photographic correction.*

We have before mentioned some difference of opinion exists as to the best position to choose for the folding over of the spectrum. The generally accepted one very decidedly leans towards the apple-green, wave-length about 5500; but we are aware, whilst saying this, that a few opticians prefer to have a much deeper green as their shortest focal length, which produces —as we look at the matter—an objective of decided under-correction.[1] The reason for this selection is, we are led to understand, that they think the combination can be used for both visual as well as photographic purposes. Others strenuously

[1] Owing to this difference of position for the folding-over point in the spectrum—this selection of the preferred colour, or that which is to have the shortest focal length—chosen by different computers, one who has an extended experience with the plate can *very readily identify* the manufacturer of the lens system under observation, this being fairly easily effected by noting the *exact* shade of colours presented along the upper and lower borders of the white line when placed as they should be, in the diameter of the field of view, oblique light being used; or within and outside the focus when the illumination is direct.

and emphatically repudiate this assertion, declaring that the performance of the objective so made for visual purposes is greatly lessened in usefulness, quality, and refinement, whilst there is not the corresponding gain—anyhow, to a sensible degree—by way of rendering it more suitable for photographic purposes, seeing that the advance towards the blue—the red photographic correction—is so small.

It may be mentioned here, for want of a more suitable place, that with those who are commencing the use of the Abbe test-plate for ascertaining the colour correction of semi-apochromatic objectives, a mistake is often made by assuming the better the combination the less the colour shown within and outside the focus, or at the edges of the white line when oblique light is used. They reason, seeing that an apochromat exhibits no colours at all of the secondary spectrum, the nearer the semi-apochromat approaches to this performance, the better corrected it must be. From what has been said, however, this has been shown not to be the case; for as a matter of fact the well-corrected lens of the type mentioned *should* show the colours of the secondary spectrum, and very plainly, too, under the different conditions mentioned. Moreover, those objectives that scarcely exhibit any colour at all under the circumstances stated are made by a "trick" well known to all computers, which consists in leaving heavy residuals in all the zones, even with the preferred colour, although not to the same extent. By their admixture these residuals form white light of a kind, which is recognised as a fog extending over the whole field, that gets rid of or may be said to hide the colour-effects, and so leads the innocent observer to congratulate himself upon being the possessor of a fine combination so "remarkably free from colour."

If such an objective—where the trick has been extensively carried out—be examined with the plate, the black lines appear positively bathed in fog, unless the iris be shut down so as to cut off the outer and intermediate zones: by which means, although the fog in a great measure disappears, it is true, the separating power is at the same time reduced very considerably also. Some of the old dry $\frac{1}{12}$ths, even by the best makers of the period, that received a large measure of praise for their "absence of colour," were made in this way, and give the

student a very good opportunity of studying this class of correction. If it should be desired to ascertain the shortest focal length of these objectives—which is rather difficult on account of the greatness of fog—it can be discovered by reducing the iris to almost a pinhole.

The summary then of these remarks, and the general conclusions for the student to hold in his mind, is that, all manufacturers taken together, the position mostly chosen by the computer for the turn-over point is very near or exactly at the wave-length 5500; and that a good semi-apochromat *should* show the *secondary* spectrum, but not any of the primary colours; and that those combinations that are vaunted as devoid of colour-effects are to be regarded with suspicion until they have passed the ordeal of an examination by the Abbe plate.[1]

We should repeat, in conclusion, that objectives corrected for photography pure and simple show with oblique light the upper border of the white line tinted with blue, and the lower one with an orange-red.

Testing the $\frac{1}{12}$ths and $\frac{1}{8}$ths for Spherical Aberration, etc.

(*b*) (i) *With direct light.*—A cursory examination is sufficient to show, as previously stated, that the whole of the field of view is not in focus at one and the same time: sharpness being limited to its central and intermediate zones. But if the objective be a good one, a touch of the fine adjustment is sufficient to sharpen up the definition of the outer zone to *very nearly equal* that of the centre and intermediate ones. If the lens be of second

[1] Although the subject has been alluded to before, it is worthy of further notice before proceeding, that the utility and benefit of having a fine image in the *preferred* colour is of just as much importance for using the semi-apochromat in photography with the aid of monochromatic screens, as it is when employing it for visual purposes: because if such be not the case, it is evident the negative cannot be a sharp one, as the monochromatic screen acts in no way to improve the image otherwise than by cutting off the damaging effect offered by, and the confusion arising from, the presence of the secondary spectrum. This offers an explanation why the older objectives very often failed to furnish good results when used—say with green light—because the image presented by them even in the *preferred* colour was so indifferent in comparison with that readily obtainable with the objective of more modern construction.

quality, no amount of coaxing in this direction with the fine adjustment will bring about any sensible improvement.

The black lines in the area of focus should look crisply defined even with full aperture (see Fig. 1, Plate II.), provided they are not so dense as to transmit no light at all; but an improvement must be expected in their definition should the iris be closed a *little*. It is just here that the difference between a first and second class objective is exhibited, for with the former an improvement is effected with the slightest touch of the iris diaphragm, whereas in the case of the latter the whole of the outer zone—as seen when looking at the back lens of the objective, the ocular being removed—must sometimes, in bad instances, be stopped off before the lines look sharply defined. The appearance of the bad definition is difficult to describe, but is best expressed as appearing like a fog lying over the whole field of view, which clears off when the diaphragm is shut sufficiently. This is due to the injurious effect produced by the outer zone spoiling the more perfect performance of the intermediate and central ones, and is due to the rays from this portion of the object glass not focussing in the same plane as the others (see Figs. 3 and 4, Plate II.; and Figs. 1 and 2, Plate III.). From this it is evident how true our contention is, that to test the performance of an objective with a numerical aperture over N.A. 1·0 fairly, it is absolutely necessary to use an immersion condenser of sufficient aperture to fill the back lens properly.

In some objectives it will be found, if monochromatic light be used, that all, or nearly all, of the fog at once disappears. This arises from the fact that the principal cause of error was due to the objective being spherically corrected for *one colour only*, and that the screen used happened to pass light of that particular colour. Sometimes, on the contrary, nothing will induce the objective to yield a really fine crisp and sharp image; there always seems to be a something lacking when its performance is compared with that furnished by a good computation. This is due to zonal aberrations. In such a case nothing but cutting down with the iris, to a very great extent too, will be of avail. It is obvious by so doing the resolving power of the objective is seriously and terribly lessened. Some of the old lenses show this fault to perfection.

(*b*) (ii) *When oblique light is used*, being arranged in the

manner previously described, the black lines should not look as if they possessed nebulous edges, or appear nebulous themselves, doubled, or fluffy.

Putting aside colour-effects, the image should look sharply defined, and the granules, if visible at all, in crisp focus (see Fig. 2, Plate II.). Moreover, when the particles that are in the centre of the field are accurately in focus, the return to direct light should *not* produce any sensible difference in their appearance—by which we mean they should not require refocussing. This we personally hold as a most important test; so much so, that we go so far as to say that there is not a good objective but what will conform to it. If only a *slight* touch is required the combination is then not so good, but if much be required it is bad. It should be remarked that if the iris be much shut then the particles in the periphery of the field occasionally appear slightly distorted, but the distortion should disappear when direct light is returned to. Some objectives of high aperture will show this defect that cannot perhaps be condemned in consequence, but we admit we like to see it as little as possible.

In the chapter upon objectives (page 71) a tabulation is given of the conditions that objectives of various degrees of perfection should fulfil. These are here again mentioned, following which will be found the appearances presented by the Abbe plate when such are *not properly carried out.* For purposes of description it is convenient, however, to take the conditions in a different order, rather than in that set forth in the table (see Addenda).

A. An ordinary low-power achromatic must be corrected for—

(1) Primary spherical aberration;
(2) Elimination of coma;
(3) Primary colour.

B. An ordinary high-power achromatic, in addition, must be approximately corrected for—

(4) Secondary spherical aberration (spherical zones) for the preferred colour.

C. Semi-apochromatics, besides fulfilling the above four conditions in a highly perfect manner, must be made free from—

(5) Primary spherical aberration for a second colour (and nearly for all colours); and

(6) Should be computed so as to give equal magnifications for all colours when used with compensating eyepieces; whilst

D. The full apochromatic must further show—

(7) Freedom from the secondary spectrum.

A (1) When focussed with a *small* central aperture, the object goes increasingly out of focus as the iris opening is moved towards the periphery.

B (4) When tested as above the object goes out of focus up to a certain intermediate zone (as the iris opening passes from centre to periphery) and returns to focus at or near the margin.

A (2) In the presence of coma *no* distinct image of an object in the outer part of the field can be obtained by any means whatever.

A (3) When no definite folding-over point can be made out, the colours obtained by oblique light are *primary ones*, and are mixed up variously with changes of focus. They are usually very pronounced both in *intensity* and *width*.

C (5) This is best detected by repeating test A (1) without any change of tube-length or correction-collar, whilst a screen or filter is used of a tint *well contrasted* with the folding-over colour.

C (6) With either direct or oblique light, the colours on the upper and lower edges of the test-plate lines should be very nearly the same right across the field.

D (7) With oblique light of any obliquity there should be only the faintest trace of colour, on the edges of the light, and this not of the usual secondary tints but more of a faintly pale blue and some sort of red.

In conclusion then we may sum up what has been said, that a fluffy jumbling up of the black lines with oblique light in use shows excessive spherical aberration of the outer zone of the objective, whereas want of sharp definition without the presence of much fluffiness or nebulosity indicates the general absence of sufficient correction. If the focus of the particles when visible varies very much when the oblique light is changed for the direct, the fault points to an unequal action of the zones and very often to a general under-correction, or perhaps to a general over-correction, or even to a mixture of both in different

zones of the objective (compare Figs. 1 to 4, Plate II., and Figs. 1 and 2, Plate III.).

Great care should always be taken not to condemn the performance of any objective for *defective spherical correction* until it has been tried successively on several of the cover-glasses of the test-plate, and, if necessary, with differing tube-lengths to a reasonable extent.

Although this applies *far more in effect and must never be omitted* when testing dry lenses, still even with the oil immersions it is well to make the trial. This is more especially the case when dealing with test-objects rather than with the Abbe plate, because the object may lie in the medium *some distance below the underside of the cover-glass*, thus increasing its effective thickness without any indication of the same to the observer, who naturally is led to believe the object is in contact with it, and hence that the stated thickness of the cover is all that needs his attention.

It remains to be pointed out that, further still, there are two curious phenomena sometimes noticeable in certain lenses. These are shown in Fig. 3, Plate III., and Fig. 1, Plate IV., the former representing the effect *outside* the focus, and the latter *within it.* Considerable difficulty has been experienced in attempting to ascertain the exact cause of these peculiar appearances. They used to be thought to arise from astigmatism, but recent considerations have led to the belief that this is not the case and that the fault arises from some non-fulfilment of the sine-law. At present, however, the subject must be considered *sub judice*, for it is not a little curious, when the phenomena are well marked, to find usually that objects in the outer part of the field of view will *not* come into focus at all sharply, but that if the "appearance" be restricted to the faintest *trace*, and that perhaps limited also, so far as can be discerned, to *outside* the focus (Fig. 3, Plate III., only not anything approaching this amount), no difficulty appears to arise in focussing all parts of the field of view alike!

Before concluding the testing of the $\frac{1}{12}$th there yet remains another use to which the Abbe plate can be put. It is to ascertain if the lenses of the combination are all properly centred. To ascertain this the lines are put out of focus, using a medium-power ocular. It matters not to do this if the objective be raised a little or lowered. Suppose it is lowered,

the purple fringes will come into view. Let the focus be arranged so that the fringes are about an eighth of the width of the black lines. Notice is then to be taken to see if all the fringes above and below the lines *are of the same width*; that is to say, if all the lower ones are the same width as all the upper ones. If they are so, the lenses are well centred; but if not, let the plate be turned around the optical axis and a position will be discovered where (in all probability) they will become so. This confirms the suspicion that one or more of the components are out of centring. Even very good objectives *may* show the existence of this fault *to a very trifling degree*, but it takes a great deal of experience to know *when the limit is passed* and the objective should be returned to the maker. But the point to recollect is, *if all the fringes are equal the centring is good.*

One caution must be given, viz. that the light and the condenser should be carefully centred, otherwise the alteration in appearance might very easily be due to an oblique light effect.

In Plate II., Figs. 1 and 2, the appearances presented by a first-class apochromat with direct and oblique light are shown, although the actual limit of the field is curtailed for lack of space.

In Figs. 3 and 4 *under*-correction with direct and oblique light is illustrated, whilst in Plate III., Figs. 1 and 2, *over*-correction has been photographed with direct and oblique light in succession. Apochromats have been chosen instead of semi-apochromats because their construction precludes the necessity of using monochromatic light.

B. Testing the $\frac{1}{12}$th Semi-apochromatic with Test-objects

Introduction

Although the Abbe test-plate is perhaps the severest trial any lens can undergo when used by experienced hands, still there are some microscopists who do not place so much reliance upon it, partly because of this great (and they sometimes say, needless) severity, and partly on account of their lacking sufficient experience for its use. They prefer, in fact, to employ some pet slide with which they are well acquainted and which presents certain markings or such-like that experience has taught them ought to be *well shown* by the best objectives, *fairly well* by moderately good ones, and *not at*

all well by those decidedly inferior. This is not the case only with amateurs, for we know that many English opticians who do use the plate very extensively, still never allow an objective to leave their testing-rooms until its performance on their test-objects has been tried.

Before proceeding to explain the appearances presented by good objectives with test-objects, we feel it our duty to call the reader's attention to several little considerations which are deserving of being carefully noticed, as they apply much more in the use of *specimens*, we think, than in the use of the Abbe test-plate.

The first is, what has been his experience and how will he know when he has obtained a critical image? If he has no idea of this, how is it possible he can test his objective to advantage before purchasing it? Perhaps he has only had the experience gained by using a two-inch, a one-inch, or a half-inch; if so, what are his prospects of success when turning his attention to the use of a sixth, and later on to that of a twelfth? The fact is, he does not know what really to expect. He soon finds out that he has much to learn, and then comes his difficulty to discover how to do so. Complaint is often made, or certainly frequently felt, that little information is given by the text-books upon this point: yet he is desirous to proceed. All these questions arise from the fact that he has no ideal at hand to compare with the image he obtains in his microscope. To try to meet this difficulty, which is a real one, Fig. 1, Plate XVII., has been made. The student should obtain a specimen of Pinnularia nobilis (selected because it is easy to obtain) mounted in styrax or, better still, in realgar. Having done so, we recommend him to try to get what appears to him is the best result possible (recollecting what has been said about altering his tube-length or using his collar adjustment to suit the cover-glass thickness), using white light first, and after that employing green. He should then compare his results with the photograph shown in the plate mentioned, and see whether it equals the illustration. He will notice that in both instances the whole field may not be quite in focus at one and the same time, as usually obtains with a first-class inch objective; this, however, he must regard as impossible; but he should also take particular notice that all parts of the field *can be made*

to come into focus if the fine adjustment be manipulated. Some objectives, especially twelfths, will focus very well in the central and intermediate zones of the field as seen in the ocular, but no focussing will produce a sharp image in the outermost one. This at once shows the combination is not the finest possible; but more will be said about this later on.

The best results having been obtained and the images carefully compared, he should upset his adjustments and reset them again and again so as to become expert at setting his instrument quickly to obtain the best results, as well as to accustom his eye to recognise them rapidly.

The tongues of the specimen (as they are sometimes called) should seem as if they were laid out upon the floor of the valve, using a little oblique light to make them do so if necessary. Faint lines ought also to be in evidence if all is in order.

When testing a twelfth, a Surirella gemma should be obtained mounted in realgar if possible (they are commercial), and he must now try to obtain the finest image possible, his former experience coming to his aid. If he compares at first with Fig. 4, Plate VIII., using green light, he will not experience much difficulty in getting as good an image; but to get as fine a result as that seen in Fig. 2, Plate XVII., he will probably find he must employ a blue-green screen and a powerful illuminant, as explained in the Addenda. It is very difficult to obtain its equal with green light.

The next is, that unless the observer be in constant practice in the art of testing objectives, his eye may very possibly lead him astray in his opinion. An object, for instance, examined by day in a well-lighted apartment rarely appears just the same as when looked at in the evening or in a well-darkened room that has been occupied by the microscopist some little time before commencing observations. This peculiarity, which is undoubtedly true, arises probably from the fact that the inherent perceptive faculty of the retina is much less when the eye is kept continuously in strong light than after a rest in subdued illumination.[1]

[1] It is thought by some observers that smoking during observations (or even immediately before commencing) is apt to spoil the sensitiveness of the retina. It is a moot point, we admit, and one that is not easy to decide, for those who express a belief in its rendering vision less acute admit that it

Then if a student examines a test-object and attempts to pass an opinion, then and there, upon the performance of a given lens, without first making a comparison with that of one or more others with which he is perfectly familiar, he courts disaster. The human mind retains the impression of visual phenomena so badly that it is only by dint of a very extensive and continued practice a professional can do so, and even he, in doubtful cases, where the fault is not so evidently patent, will always prefer to compare the image with that produced by other combinations before he gives his final opinion.

Another pitfall is the exact state of illumination at the moment of observing, which is of more paramount importance with a test-object than with a test-plate. A fortunate and exceptionally fine arrangement of the light *may* show up the details in a specimen that even a poor lens may reveal, so as to "make believe" it is a better one than was expected; whereas just the reverse—which is more to the point—a bad lighting may quite spoil the fine effect of a good combination so as to impress the microscopist very strongly against it. Now all these erroneous judgments are cleared by comparison with the performance of other objectives; for if the lighting be extra good for the one, it is probably so for the other, and *vice versâ*. This remark very strongly applies when the limit of resolving power is being approached, such as the breaking up into dots the well-known lines in Amphipleura pellucida, say, with green light. The *smallest* amount of change in the obliquity of the light and so on may make a serious difference in the apparent performance of the objective. But if the performance is comparative with that of other lenses, a faulty arrangement of details may, and probably will, affect each one in its turn alike. But a word of caution must here be given. Owing to the fact that all objectives, even of the finest type, suffer from zonal defects, although very likely only to a minute degree, still on this account it should not be forgotten it is quite *within the range of possibility* that the exact lighting so suitable to one lens might not *ex necessitate* be so suitable to another. It is advis-

does not do so at *all* times. It is merely mentioned here in case the reader may be one who is, or can become, affected in a prejudicial manner from this cause, so that he may avoid misjudging the value of any lens owing to his sight being temporarily affected.

able, therefore, when testing a lens, if its performance be not quite so good as any of the others, to try *moderate* changes in the details before expressing a final opinion. The state of the eye, too, is a factor which must not be overlooked: we mean with respect to fatigue. There is no question that the powers of perception rapidly go off as the eye grows weary, especially if the attention has to be very greatly strained at the same time and the light be very intense. As an instance, we know nothing so fatiguing as the examination of the faint lines in the "rulings" on glass, which used to be often employed. The eye is strained, and the attention also, to see whether the objective will or will not separate a given series. In our experience, so great is this strain that we cannot examine more than two or three combinations with this object in view without a somewhat protracted rest! So with other test-objects it is true, but never in our experience to such an extent. As the eye rapidly grows weary —especially if the observer be otherwise rather tired—it is well to change about the series of objectives, the one to be tested with the others that are well-known, so that the effect of this growing fatigue may become insensible and lost by being distributed over the whole group.

Prejudice, too, has to be dealt with. If the microscopist is prepared to expect to see something very exceptional (perhaps, in the case of a beginner, what is even beyond the limits of possibility with the lens in question) and fails to observe it, disappointment is felt, and a feeling akin to disgust or dislike is generated. On the other hand, if the mind has been led, from some cause or other, to expect a poor exhibition, an indifference is engendered which leads to a careless adjustment of details with the light, focus, and so on, so much so that the objective is not really fairly treated. It is more and more evident then as we proceed, that, for the student anyhow, direct comparison with other objectives that are recognised to be good ones by those who *do* know, is a far better plan and one which leads to far better results, than to make the attempt at forming an opinion by comparing the performance with *a mental recollection* of that given by some other lens at some other time, and perhaps exhibited by some other person.

Lastly, an opinion should not be hastily arrived at by the examination of *one* pet specimen. It is quite possible, and

indeed a well-known fact, that one particular objective *may* show a particular object in a particular slide, with a particular index of refraction of the mountant, better than any other combination, but which, when tried on several test-objects and the plate, may lose its high estate. This peculiarity arises from the simple fact that the structure of the individual specimen, accompanied by a happy and fortuitous combination of the details mentioned—*all acting in concert*—may just happen to suit the computation in such a manner as to produce an effect that will lead the microscopist to form quite an erroneous opinion as to the *general* value and perfection of the objective in question. When, however, an opinion is based upon the performance of the combination with *several* test-objects as well as the Abbe test-plate, such a misleading effect is not likely to assume so great a value, or anyhow, will be effectively discounted by the general performance of the lens taken as a whole.

Before proceeding further, the reader must now prepare himself for a somewhat serious reflection about to be cast upon the performance of both the semi-apochromatic and apochromatic combinations to which at present scarcely any attention has been given. It is this. When using either class of combination *at full aperture*, and with a *full solid cone* of critical light from an oiled condenser of approximately the same N.A. as that of the objective upon a test-object—such as, for example, a Navicula lyra—no combination is in existence that we know of which will yield a perfect image, or indeed anything approaching thereto. The diatom is seen to be so bathed in fog that no details, or scarcely any, are visible. This arises from imperfections, speaking in general, that are still present in the outer zone, and left uncorrected even with these otherwise magnificent combinations. It seems hard to say this, but it is the truth nevertheless, and the fact is too well known by computers and opticians, as well as by microscopists in general, to allow of dispute. That the art of the optician in constructing, and the talent displayed by the computer in devising objectives of recent date—principally owing to the advent of the Jena glass—have vastly improved the performance of this outer zone there is no denying, for the area in question will now furnish quite a good image if used by *itself* as obtains when oblique light is employed; but the point is that when this zone *is used in concert*

with the others the rays it subscribes still ruin the definition of the final effect. That this is so is proved by the simple fact that when such contribution is cut off by a suitable closing of the iris diaphragm, the true details of the test-object immediately flash out all of a sudden, the fog entirely disappears, and excellent definition is at once obtained. An additional experiment dealing with the matter in another way may be tried. A Traviss's Expanding Stop, or a wheel diaphragm (cut out of black cardboard similar to those made in metal for producing dark-ground illumination—see page 173), should be placed beneath the substage condenser, its occluding portion (or box of the wheel) being the exact size to stop off the central and intermediate zones of the objective as seen when looking at its back lens, the ocular being removed.[1] When the image formed in this way, that is, entirely by the rays issuing through the outer zone, is critically compared with that produced by the opposite method—the conjoint action of the middle and internal zones produced by stopping off the outer area by closing the iris a sufficient amount—there can be no doubt in the mind of the observer which is the better of the two. The poorness of the definition of detail, so pronounced in the first instance, is conspicuously absent in the second. Hence there can be no hesitation in affirming that the falling off in the definition and the introduction of fog, both of which are present when the full aperture is used with objects like that suggested, may be justly attributed to the "spoiling or deteriorating effect" of adding these rays from the outer zone to those derived from the two inner ones already mentioned. The improvement then to be effected in the lens of the future, if such can ever be attained with our existing knowledge, must lie in so improving the performance of this offending area of the objective, that when its contribution is added—the objective being thus used at full aperture—the combination shall perform as well as, if not better than, it does now, when the inner and central zones are alone being employed. Then, and not till then, can we really call an objective perfect.

We might mention however in passing, it has been often asked, if the rays from this zone be of such a deteriorating nature when added to the others, why are they not always cut

[1] This is using what is called annular light. See Addenda.

off by a suitable diaphragm placed at the back of the combination? A moment's consideration, however, especially if the student has read or will refer to the chapter on numerical aperture, will suffice to show that such an idea cannot be tolerated a moment, for by so doing the resolving power of the objective would be thereby enormously reduced, and the advantage gained by the use of oblique light entirely lost.

But the querist may add, what can be the advantage of employing this oblique form of illumination, seeing it demands the selective employment of the bad area in question? We here reply, that whilst allowing the "spoiling effect" of the rays coming from the outer zone *when added to those issuing from the central and intermediate ones*, still it must never be forgotten what we have already said, that in the present day very fine images are produced by the zone in question *if employed alone.* This we may add will be better understood when we come to talk of the exhibition of dots in Amphipleura pellucida, only about $\frac{1}{100000}$ of an inch apart, which cannot be shown *at all* by the conjoint action of the inner and intermediate areas of the lens *if alone employed.* The question then of retaining this outer zone will now immediately be settled; but the matter will be better understood if the chapter upon the use and theory of oblique light has been carefully studied. The reader will gather from this the improvement in the performance of the outer zone, as exhibited by objectives of modern make, is very marked, and as it varies in different manufacturers, so its better performance in one lens in comparison with that exhibited by another, offers one of the means of distinguishing first-class computations from those of inferior design; but it remains to be hoped that eventually improvements will be effected of so high an order, that a combination will be designed and made that will work at *full* aperture, and in such a manner as to exceed all expectations of the moment.[1]

It is evident from what has been said, at the present time then we must content ourselves with examining objectives as we find them. For this purpose, in addition to the plate

[1] Some attention, especially of late, has been given to the use of a Nicol's prism above the ocular, or a polariser beneath the condenser, as a means of increasing the defining power of the objective. The matter is discussed in the Addenda.

previously spoken about, certain diatoms are employed, being selected for a threefold object: to test the performance of the combination as a whole, to satisfy ourselves as to the rendering of images by the outer zone only, and to the definition produced by the combined action of the middle and internal ones alone employed.

Test-objects

Amphipleura pellucida.—We use this diatom to test the performance of the objective as a whole, and also as one to indicate the rendering of the image by the selective use of the outer zone only.

When a first-rate objective is employed in conjunction with a × 6 compensating ocular, and a full cone of white critical light from an oiled condenser of approximately the same N.A. as the objective, the transverse lines about $\frac{1}{100000}$ of an inch apart should *just be visible*; not distinctly rendered, as in Fig. 2, Plate IV., for that requires oblique light, but certainly distinctly visible. We have never found this test fail us, and we may honestly say we have examined the performance of a very large number of $\frac{1}{12}$ths, representative manufactures of all the best makers in the world. But to make this test a valid one, two precautions are to be observed: first to have a good specimen, and secondly that the light shall *not be in any way oblique*. With respect to the first, it is best for the student to obtain the assistance of some friend who has a well-tried and not-found-wanting objective, to pick him out a specimen that is well marked. They are of two kinds. One is an exceedingly small variety, where the lines are extremely difficult to see, and the other a larger type, where the lines, although not more than about $\frac{1}{100000}$ of an inch apart, are still nevertheless well marked and absolutely distinct. It should be mounted in realgar. Concerning the second, seeing that any $\frac{1}{12}$th objective, if it be worthy of the name, will show the lines with oblique light, the care to be exercised that the illumination is *distinctly direct* is simply a precaution to prevent a poor combination—by this accidental and unfair assistance—being mistaken for a good one. We admit the test is a severe one, but it has a remarkable use in disseverating the good from the second-class objective, not only by *showing* the lines, *but in the distinctness with which it does so.*

A good combination will depict them almost perfectly, if not quite so; but a second-class type of manufacture, while showing them, it is true, fails just in that particular of furnishing a neat and crisp definition which the observer will soon learn to immediately recognise. A low-power ocular should be used.[1]

To use the diatom for testing the performance of the outer zone, a specimen must be selected by the friend before mentioned, that has been ascertained by him to show the dots well and discreetly defined, when sought for in a particular manner. To make what follows intelligible, the reader is requested to read the remarks upon obtaining and using oblique light in the chapter devoted to the purpose. After doing so, the objective under examination should be oiled to the cover, and the slip to the condenser as there explained, and the direction of the oblique beam of light so arranged that the two diffraction-spectra, in company with the direct beam, shall lie in the field at *one and the same moment.*[2] If now green light be used, the

[1] This test is also of great service to ascertain experimentally whether any component of an apochromatic system is commencing to disintegrate, such decaying being a well-known drawback to the use of this type of combination. It may commence at any time—quite unknown to the user—or may not occur for years. If then an objective suddenly ceases to show the lines, it may be taken for granted, almost for certain, that trouble has commenced in its components. A word of caution must, however, be added. The eye should never be exposed to a bright light immediately before commencing operations: doing so is a frequent cause of "missing" the lines, and a lens in consequence may, quite incorrectly, be condemned as at fault, when the eye is really the culprit.

[2] One might call attention here to the fact that in doing this, the field of view may rapidly darken because the flame image may not entirely cover the whole area over which the aperture of the diaphragm is being shifted to find that particular position where the dioptric or central beam, as well as the two spectral beams, shall lie in the field of the back lens at one and the same moment. The immediate effect of this is that the mirror has to be altered for every shift of the central beam, which is troublesome. The difficulty can usually be met by moving the *object* around the axis, instead of *interfering with the substage arrangements.* The only drawback to this is when the eye of the observer is astigmatic, by which is meant, sees objects in some meridians better than in others. It may happen that the best position for the object to lie in (to bring about the ideal of having the three beams, two spectral and one dioptric, in the area of the back lens at one and the same moment) is that particular meridian *in which the astigmatic eye sees worst.* If an observer be astigmatic then, this plan suggested of moving the object instead of the iris aperture loses much of its utility.

dots should be sensibly visible, as shown in Fig. 5, Plate XII.; but their *perfection* of definition is in direct accordance with the aperture of the objective and the beauty of the correction of its outer zone, and nothing but a sensibly perfect computation will show them, as in Fig. 1, Plate VI., in Figs. 5 and 6, Plate XIII., and in Fig. 2, Plate XV., the photographs being taken at different magnifications to furnish the reader with an idea of what to expect to see under the circumstances, when blue light is employed with different amplifications. Practice will enable the skilled tester of lenses to form a shrewd opinion as to the excellence of the correction in question by a mere glance, but one who is but a learner will do well to compare its performance with that of the master lens before giving his opinion. Indeed, so true is this remark, that even many practised observers will never deliver any judgment without first comparing with another objective they know performs well. To obtain blue light it is recommended to employ the prismatic illuminating apparatus (page 170), or to use the blue-green films made by Kodak, which are exceedingly good, and which are fully explained, with the illumination required, in the Addenda.

Navicula lyra (Plate V.).—This diatom is one for testing the performance of the central and intermediate zones of an objective. By this we mean, when using a full cone of the same aperture as the objective the iris must be closed to cut off the outer zone. *The amount needed to produce really fine definition denotes the superiority of one objective over another.* Moreover, this test-object is an excellent one to show the chromatic correction of a semi-apochromatic objective, the folding-over colour being easily seen as well as that of the longest focal length. It also furnishes a useful object to show the complete elimination of the *secondary* spectrum in an apochromatic. See explanation to Plate V.

It is a matter well known to microscopists, that with different planes of focus two leading appearances are presented by all diatoms that have a recurring structure composed of dots. These are called the "black-dot" and the "white-dot" image respectively. Some objectives seem always to show one image better than the other—that is, the black-dot effect or the white-dot image, whilst another seems to be able to render both equally well. It is a matter of some difference of opinion which

betokens the finer computation. To decide would involve a theoretical discussion upon the formation of these different representations of the diatom's minute structure that is foreign to this part of our book, but we have a leaning ourselves—taken for what it is worth—to the black-dot effect being indicative of the finest computation, and many are in accordance with us in this opinion.

With the Navicula mentioned, the image should be very sharp and perfect *if the outer zone of the objective be cut off*, and the combination is indeed a poor one that requires much, if any, further closing down with the iris before perfection is arrived at.

Pleurosigma angulatum.—This diatom is a great favourite with opticians at home as well as abroad ; but its use is rather difficult, for to make it of real utility a very considerable amount of practice and experience is necessary. One thing that complicates its use—anyhow, at first—is the fact that it presents *several* "appearances," according to the exact focus that obtains at the moment. It may appear, for example, as if covered with circumscribed minute black dots similar to those shown in Fig. 1, Plate XIV., called indeed by many "the black-dot focus" ; conversely there is what has also been questionably termed "the white-dot effect," where little perfectly white hexagonal areas are seen bounded by six very black and accurately defined walls, as shown in Fig. 2, Plate VI. ; whereas, lastly, a third appearance—especially noticeable when the valve is mounted in realgar, and which we ourselves regard as the *true* rendering of this difficult object—is capable of being seen when the focus is made such as that depicted *in the centre* of Fig. 3, Plate VI. Here each little white dot appears as a minute and distinct *perforation* in the pale-coloured substance of which the diatom is made ; there is no six-sided white space and no black dot, both of which we personally believe are really due to very exceedingly minute errors in focussing. More especially do we say this because certain curious false images, due probably to diffraction phenomena, are capable of being seen in the black hexagonally arranged boundary walls above referred to if care be exercised to show them, and the magnification be sufficiently great. For this purpose an enlarged *negative*, obtained with an excellent 2-mm. apochromatic by Leitz, is shown in Plate VII., where several can be plainly seen in the boundary walls which

are white in this particular instance, because the enlargement is that of a *negative photograph* which shows them better than a positive one. But, however, this is not the place for discussion of the Pleurosigma and its many appearances, so we pass on to the use of the diatom *as a test-object.* Seeing that no objective will render a good image of any of the different images with full aperture, opticians, we believe, do not employ the diatom mounted in realgar, but dry and burnt on to the cover-glass, or mounted in Canada balsam.[1]

With this class of specimen the best objective is the one that shows *the most perfect rendering of the black dot.* It should not be *grey*, but black, and the smaller the amount of cutting down by the diaphragm required, the finer the lens.[2]

It is very evident then, seeing this is what may be called a quantitative test, it requires a great deal of practice, and many objectives must be compared and seen before the microscopist can trust himself to form a really good opinion. It will be found, too, that with the diaphragm very considerably closed, there are *several* black-dot appearances and several hexagonal images also, layer upon layer! This is a disquieting matter, and has given rise to a good deal of discussion; but the only explanation that seems to hold its own against the wrath of opposing theorists is that furnished by the papers upon the subject by Mr. Conrady, to which the reader is referred for further information.[3]

When viewing a specimen mounted in realgar, as previously mentioned, we think the effects are all emphasised very strongly; but the exact focus that images the true white appearance, as

[1] It will be seen then by this means a 1·40 really performs at an aperture of 1·20, because on reference to the chapter on the use of the substage diaphragm, it will be understood that with an N.A. 1·0 *condenser* used with an objective of greater aperture, the *working* aperture is found by adding the apertures of the objective and condenser together and taking the mean. Thus speaking of a 1·40 objective, this becomes 1·40 + 1·0 = 2·40, the mean of which = 1·20 = the real working aperture of the objective under consideration.

[2] It is not a little curious that some otherwise very good objectives certainly show the white "effect" *better than the "black dot."* After many years of observation, however, we feel bound to assert that, on the whole the better the combination, the better the rendering of the black-dot effect, *no matter the appearance*, within reasonable limits, of the white one.

[3] *Journal R.M.S.* 1904, pp. 610-33; 1905, pp. 150-2; 1905, pp. 541-53.

we are pleased to call it, Fig. 3, Plate VI., shows up the delicacy of the correction of the combination to a remarkable degree. So much is this the case, and so delicate a test do we believe it to be, that it is absolutely necessary to carefully adjust the tube-length—even with a homogeneous objective. This careful adjustment applies also when obtaining the best image of what is termed a "postage-stamp fracture" (often observable in a broken valve that has lost its back portion), such as that shown in Fig. 3A, Plate VI. These two realgar images will sometimes be of great service in assisting the microscopist to distinguish the performance of two high-power objectives, otherwise so excellent as to resist comparison even with tests usually considered as more severe, like Amphipleura pellucida, Synedra capitata, or Nitzschia singalensis. See explanation to Plates VI. and VII.

Coscinodiscus asteromphalus is a favourite with some. As this diatom is saucer-shaped, it is impossible to obtain a large area that is flat enough to furnish a uniformly focussed field, save of a limited and circumscribed nature.

Midway between the centre of the "saucer" and its periphery is perhaps the best spot to choose for an examination, and such is shown in Fig. 1, Plate VIII. But the diatom has two very distinct and very different planes of focus, only one of which is illustrated in the previously mentioned photograph. It is possible, however, inasmuch as the little saucers are very often not flat or of equal thickness—*at their peripheries*—to choose a specimen which, if focussed at its centre for the purpose, shall show both planes simultaneously, as given in Fig. 3, Plate IV., of a variety of Coscinodiscus closely allied to the Asteromphalus called the Opthalanthus (see explanation to Plates IV. and VIII.). To obtain either of these, however, it is imperative to cut down the aperture extensively—indeed, very often to the central zone—hence we ourselves do not regard the test-object of any value to indicate the performance of the outer and middle areas of the objective, but of the central one only.

Frustule saxonica—often called *Vanheurckia crassinervis*—is a magnificent diatom for testing the performance of the outer and intermediate zones. With full aperture and direct light the transverse lines should just be visible, and their presence immediately emphasised by the use of a little oblique light.

When the illuminating arc (as explained in the article upon oblique light previously referred to) is turned around the optical axis, a moment will arrive when the lines are broken up into extremely minute dots (Fig. 5, Plate VIII.), almost vieing in smallness with those of Amphipleura pellucida. The sharpness with which these are seen, especially when employing white light, offers a means of distinguishing good from inferior combinations. The specimens should be mounted in realgar, and as they were easier to obtain than Amphipleura, some have in the past preferred this test-object. See explanation to Plate VIII. For the assistance in definition that some think is afforded by the use of a polarising prism, see Addenda.

Surirella gemma is a useful and easily obtainable diatom. It should be procured flat and well marked, mounted in realgar. It affords a fine opportunity for witnessing the rapid alteration from black to white-dot effect, by an incredibly small change of focus (Fig. 4, Plate VIII., and Fig. 2, Plate XVII. See text to Plates). A fine objective shows these dots exceedingly well depicted, crisp, and sharply defined, and hardly any shutting of the substage iris ought to be necessary. If much be required before a good black-dot effect is obtained, the combination is a poor specimen of the optician's art. It is best seen with blue light. See explanation to Plates.

The Podura scale [1] used to be a favourite in England for testing the colour correction of an objective. We do not like it for that purpose, as we think it may be misleading, seeing that it always requires the outer as well as the middle zones cut off before a well-defined and perfect image is obtained. Hence we merely regard its use as a test-object for ascertaining whether the centre zone is well corrected. In good specimens used for this purpose, the white marking lying lengthways down the centre of each so-called "note of exclamation," when closely examined and carefully focussed with a good objective, should show a well-defined constriction around its neck; and further still, the white interior should not end abruptly, but be seen

[1] Most of these specimens are of old date, and consequently mounted with rather thick covers. The microscopist is warned of this, for several 1/12ths will not work through this thickness, their "working distance" being too small. It is usually necessary, before obtaining a well-defined image, even with homogeneous objectives, to considerably shorten the "draw" to get over this extra thickness of cover.

prolonged a long way down the note, delicately tapering off until it almost imperceptibly fades away. This may not be visible with all the "notes," but should be seen in some with careful focussing (Figs. 2 and 3, Plate VIII.). See explanation to Plate.

Although we have mentioned what perhaps may be called some of the *classical* test-objects, still the following have been used by us with no little profit, so we enumerate them with their peculiarities, in the hope they may be of considerable service to those interested in the subject.

Nitzschia obtusa.—In our experience, this is a difficult diatom to obtain that is well marked. When a good specimen is examined with a first-class apochromatic and the use of oblique light, the transverse lines, which are exceedingly close together,[1] should be resolved into dots closely packed side by side. Some care is needed in getting all the requisite spectra into the field so that the dots shall be well seen. A good objective should show these with ordinary white illumination, but the extra power of resolution afforded by the use of green light is needed for very exquisite definition. Fig. 1, Plate IX., was taken with a Reichert 2-mm. apochromatic of great excellence. See explanation to Plate.

Brebissonia Boeckii is a very difficult diatom to resolve, in saying which we do not refer to the showing of the striæ, but to the resolution of these striæ into exceedingly minute dots. Inasmuch as this diatom is anything but flat, and the details are so small, the microscopist must not expect to see many of the dots in focus at one and the same moment. It is an exceedingly difficult object to photograph. Fig. 2, Plate IX., was taken with a very superior 2-mm. apochromat by Leitz. See explanation to Plate.

Synedra crystallina.—This diatom is fairly easy to see in interrupted lines; but to show these broken up into sharply defined quadrangular dots requires an exceedingly well-corrected objective. Fig. 3, Plate IX., was taken with a remarkably fine 1·5-mm. apochromatic by Koristka. See explanation to Plate.

Cymbella gastroides (*small* variety) is an excellent test for the colour purity of a combination. The dots should be shown

[1] The late Van Heurck says the lines are "fine 26 or 27 to a c.d.m.' 1 c.d.m. = ·0003937 English in. = 10 microns. = ·01 mm.

as more or less rectangular nearly everywhere, save in the central portion of the diatom and near the median raphé, where they taper off to a point well seen in the photomicrograph. These little markings should "stand out" very distinctly from the background and be free from haze, even when using white light. Fig. 4, Plate IX., was taken with an excellent Hartnack 2-mm. apochromatic. See explanation to Plate.

Navicula Smithii.—The true nature of the zig-zag rows of dots was not discovered till the advent of first-class homogeneous objective (Van Heurck). When using a combination that is sensibly perfect, these dots should be very *distinctly circumscribed*, such as shown in Plate X., which was taken with the 1·5 apochromatic by Koristka above mentioned.[1] See explanation to Plate.

Navicula firma is a curiously marked, rather flat diatom; the peculiar shape and delicacy of the dots, which are inclined in places to be ovate rather than round, require a first-class objective to render them distinctly, even with oblique white light. Their separation (both as white or black dots) should be distinctly seen, but it requires a fine combination to show them as seen in Fig. 1, Plate XI., taken with a 2-mm. apochromatic by Reichert, using green light. See explanation to Plate.

Epithemia turgida is a test-object in which the dots are very plainly visible; but their exact shape in their doubly arranged rows is a matter we have never been able to be quite certain about. When slightly *out of focus*, as in the right-hand portion of Fig. 2, Plate XI., they look circular, whereas in the centre of the specimen where they look sharp, they appear almost triangular; but at a third plane, as shown in the left-hand end of the photograph, where they are just going out of focus, they appear for the most part round! After paying some attention to this matter, we lean towards the opinion that they should be always shown as circular, although it is difficult to explain their triangular appearance in the central portion. A photograph

[1] Mr. A. A. C. Merlin, of the British Consulate, Volo, Greece, whose power of sight for discerning secondary markings is envied by many microscopists and whose patience and care in observing difficult objects are well known, calls attention to the fact that a larger variety of this diatom shows *additional* markings. The late Dr. Van Heurck stated the photograph shown in the Plate is characteristic of the smaller type.

showing the three effects has been chosen to better explain the text. The neatness with which these effects can be disseverated and the general sharpness of the whole afford a guide as to the excellency of the objective. The photo-micrograph was taken with an exceedingly fine Leitz 2-mm. apochromatic. See explanation to Plate.

Cymatopleura solea.—In the floor of this little valve, which is very narrow, may be seen, when carefully searched for, if necessary with the aid of oblique green light, a series of closely arranged transverse lines. They appear as if roughly ruled, and engender the belief that they could be broken up into dots, but we have never been able to do so, however. They are very delicate, and are extremely difficult to photograph on account of their great transparency; any diffusion of focus in the objective, and they may be entirely invisible. Fig. 2, Plate XII. (Further details of interest have been found and photographed by Messrs. Herbert & Blakeley respectively.)

Navicula rhomboides.—We have no great belief in this diatom as a test-object save in one point, viz. that the little black dots should look distinctly circumscribed without fuzzy edges. This betokens, in our opinion, a finely corrected lens, and is well seen in Fig. 1, Plate XII., taken with the irreproachable 3-mm. apochromatic by Zeiss. This diatom well exhibits the colour correction of a semi-apochromat, hence is very useful for this purpose. See explanation to Plate.

Eupleuria pulchella (Arnott).—This diatom, which does not seem mentioned by many books devoted to the subject of the Diatomaceæ, when examined by direct light appears as an exceedingly transparent object, giving but a faint trace of secondary markings with full aperture. With very carefully adjusted white oblique light (of great obliquity) at a certain meridian, very pronounced, extremely minute points may be seen studding the quadrangular sections of which the object seems composed, but they are better shown with green illumination. Excepting with very well-marked specimens these can be easily overlooked, and they are especially troublesome to photograph. Fig. 3, Plate XII., taken with an excellent 2-mm. apochromatic, manufactured by Hartnack. See explanation to Plate.

Nitzschia sigma is yet another test-object that requires a good

objective. We do not regard it as one requiring the extreme refinement of the use of oblique light (as the dots are fairly large), but as one when the perfection of the *central and intermediate zones* of the objective are more especially under examination. The dots should be pronounced and uniformly defined, no blurring or running together visible, and they should stand out well from their background, as shown in Fig. 1, Plate XIII. Photographed with an apochromatic $\frac{1}{12}$th by Powell & Lealand. See explanation to Plate.

Synedra capitata.—This diatom is very common, being found "almost everywhere," according to Van Heurck, and its length, he adds, varies from 20 to 50 c.d.m. (see Index). The transverse striæ, about $\frac{1}{200000}$th of an inch apart, are easily seen with a $\frac{1}{12}$th, but to break up these lines into segments, usually about fourteen to sixteen to each line, is somewhat difficult. A finely corrected combination should show these secondary markings as distinctly rectangular in form (as in Synedra crystallina), having their greater measurement lying parallel to the long axis of the valve—that is, transversely to the direction of the striæ. Owing to their unusual shape, some refuse to call them "dots" in the ordinary acceptation of the word. Green light will resolve them fairly well if used strongly oblique; but to see them really well and neatly defined, oblique blue-violet illumination (maximum transmission about 4700 tenth metres) and a powerful illuminant are necessary. These secondary markings can be made to appear as black or white "effects" according to the focus, and both are shown in the photograph, Fig. 2, Plate XXI. Those in the centre look black, whilst those at one end are white, the two "appearances" in one illustration being, of course, due to the specimen not being quite flat.

We have never been able to resolve this diatom with a $\frac{1}{8}$th, the nearest approach thereto, in one instance only after trying several objectives, apochromatic and others, being a faint exhibition of one or more "dots" near the median raphé.

It should be noted that these secondary markings are formed in this instance by the breaking up of the lines—as usually obtains with most diatoms—and are not due to a resolution of the spaces between them, as some think to be the case with Navicula cuspida.

From what has been said, it will be readily seen that Synedra

is a test object for the outer zone of a $\frac{1}{12}$th only, as oblique light, we have always found, is imperative. The image should be clean and sharp.

Pygidium of the Flea.—This specimen can be used for a $\frac{1}{12}$th, but is mostly employed—and is perhaps more suitable—for a $\frac{1}{6}$th, amongst the test objects for which it is fully described.

Remarks

on Testing Apochromats of N.A. 1·30 to 1·40 of a Focal Length varying from $\frac{1}{8}$-in. to $\frac{1}{15}$-in.

with the Abbe Plate

It is customary, when speaking of apochromats, to say the images formed by them are *absolutely* colourless, and this is *practically true* when dealing with test-objects of all kinds if the computation be of the best type, but with the Abbe plate the statement requires qualification. We believe Professor Abbe himself, in his scientific remarks upon the achromatisation of his apochromatic system, only claims that seven-eighths to nine-tenths of the secondary spectrum are entirely and completely eliminated. When using the Abbe plate, seeing that it affords an extremely delicate test-object, it is not difficult to understand the possibility that it might be able to show this residual one-eighth or one-tenth of which we have just spoken. It seems a matter of very considerable difference of opinion amongst those qualified to speak, whether this trace of colour should be possible to be seen, or whether it would be too faint to make its presence felt. Some consider all colour should be absent, even this microscopical trace of tertiary spectrum; whilst others hold if such be absent, the absolutely pure colourless effect is really due to a trace of spherical haze in one or more of the zones which hides, obliterates, or covers the colours, rendering them unobservable by the eye. When the "powers that be" differ, it is hard to lay down any law upon the matter. We have tested the apochromats of very nearly, if not of absolutely every maker of repute in the world, and we find some do show this trace with the plate, whilst others do not. Two specimens even from the same firm of opticians—both magnificent representations of the manufacturer's art, furnishing irreproachable images of all classes of test-objects—actually differ to a *very* slight degree in

this particular respect when used on the plate. After repeated examinations we have sometimes thought (although not always perhaps) the better image—if one can be called better than the other—is that produced by the minute colour-rendering combination (in contradistinction to the colourless one), which goes rather to support the theory that such residuals of colour *should* always be visible; but it is obviously a very difficult matter, with such a degree of refinement of which we speak, to be quite positive in one's assertions. At this point then we must leave the question of chromatic correction for those better able to speak than ourselves.

As regards the definition of the particles of the black lines of the plate, nothing should be left that is desired. Of course the field is not flat any more than with the semi-apochromat, indeed, sometimes we have thought the flatness is more curtailed; but the absence of all fluffiness, even when more than a moderate amount of oblique light is used, and the absence of all doubling of the lines and loss of definition when returning to direct light after having focussed with oblique illumination, should be conspicuous and complete (see Figs. 1 and 2, Plate II.).

Remarks

on Using Test-objects for Definition, etc., with the Apochromatic $\frac{1}{12}$th, etc., of 1·30 to 1·40 N.A.

When comparing the images of diatoms produced by the semi-apochromat and the apochromat, there is one remarkable difference noticeable if the light be not monochromatic. The diatom itself appears tinted in exact accordance with the turn-over colour for which the combination is corrected; hence in most cases it appears distinctly apple-green. With an apochromatic, however, that is properly corrected, the little valve is rendered perfectly white and colourless; an object beauteous to behold. It is this that makes their use so coveted by the enthusiast in this line of research; but whilst saying so, still we must in duty point out what we have mentioned before, that if the microscopist be content with monochromatic-green illumination, the difference in the actual *details and definition* of the object, as shown by the really well-corrected semi-apochromatic, is not always so immediately apparent and distinguishable from

that afforded under similar circumstances by its more expensive rival. It may astonish some to hear, with specimens of manufacture of both types of lens-construction by the same makers which we have in our possession, that if the images are compared in this manner with monochromatic *green* light *upon the dots in Amphipleura pellucida*, a great difference in performance is not *immediately* apparent; and that some care must be given before it *may* be detected.[1] Still there are occasions when the use of green light is not desirable; even the advantage gained in the separating power imparted to a lens thereby may not entirely compensate for the monochromaticity of the light, for it certainly dulls the illumination very sensibly, unless a very high-power illuminant be at hand. Then the expensive lens makes its value felt, and resort must be had to its use.

Hence it arises that we have recommended elsewhere in this book for ordinary purposes, with or without green light, the semi-apochromat as fulfilling all or nearly all requirements; but that when diatoms are studied, or a court of appeal is desired, then the apochromat should be employed without a shadow of doubt. The evidence of this and the justness of this remark are not far to seek, and indeed should never be forgotten, namely, that the care expended upon these objectives on their zonal and other corrections *should* of course all add to the perfect manner with which they delineate the delicate details of any minute object, and that it is only reasonable to expect something more with one lens than the other, seeing that its price is nearly four times as great! See Addenda—the different corrections for objectives.

Nothing further need be added with respect to the performance of this type of objective with Amphipleura than the points mentioned when dealing with the semi-apochromat. The lines at full aperture, owing to the absence of the secondary spectrum, should be exceptionally well seen, and of course more clearly apparent from the same cause. The dots should look exceedingly well defined, but, as we have already pointed out, when using monochromatic green light there is little difference in the

[1] This is not true when blue light is used, for here the semi-apochromat fails, owing to its "preferred colour" being apple-green. An improvement is, however, at once effected if the tube-length be changed. With apochromats, of course, it is here they immediately assert their superiority.

performance of the semi-apochromat and the true apochromat if they be of equal merit. There is one difference, however, of which we have not spoken, and that refers to the difference in the brilliancy of the two images. The difference is very striking if white light be used, and can be noticed also with monochromatic illumination. It is only to be expected, however, and is not what may be called a fault, seeing that the image is formed by the union of three colours in the case of the apochromat in contradistinction to the more restricted union which takes place with the semi-apochromat.[1]

Navicula major.—This diatom, which is very common and easily procured ready mounted in styrax—occasionally in realgar—is little known, so far as we are aware, as a test-object for the outer zone of a $\frac{1}{12}$th. If viewed with central cone, the "tongues" appear clean and sharply defined, much as seen in Pinnularia nobilis, Fig. 1, Plate XVII. Using oblique green light and an oiled condenser of sufficient aperture, a good immersion $\frac{1}{12}$th, however, should now show that each of the "tongues" have several striæ upon them, and that linear markings are frequently to be seen upon the diatom itself between the tongues. If a powerful blue-violet light (maximum transmission 4,700) very obliquely cast be employed, instead of the green, all the markings in question are seen plainer still.

It requires some searching to find a perfectly flat and well-marked specimen, and one more especially which with oblique light does not show some blotchy blemishes, very often quite invisible with direct illumination. Further, it is not quite easy so to arrange the light that the markings up and down the entire diatom, or the portion in the field, are all in focus at one and the same time. An attempt has been made in Fig. 3, Plate XXI. On examining this photograph with a hand-magnifier, the markings are shown nearly everywhere on the tongues and in several positions between them. The student in arranging his oblique light must shift his mirror very carefully, so as to get the small portion of his condenser in use as brilliantly filled as possible, otherwise he will find the field too

[1] The student should bear in mind that no matter what optical system be employed, the image of a source of light *can never be so bright as the source itself*: it can vary, however, and in this case it is in favour of the apochromat.

dark to see satisfactorily. Different objectives will vary in their performance, and the observer will require to be very patient to obtain the best results, for they are difficult to obtain satisfactorily.

Navicula lyra should show no *colour*, and the dots ought to look magnificently defined and of course of a pure white colour; but it should be recollected to use as full a cone as possible (that is, as large as may be consistent with fine definition), for an over-closing of the iris may very readily produce a tinted effect in these minute little objects otherwise not apparent with a larger illuminating cone (Plate V.).

Podura scales should show the constriction about the neck especially well because of the absence of the secondary spectrum, and the tapering off too of the white interior of the note, to which allusion has already been made in dealing with the performance of the semi-apochromat, should be especially well seen (Figs. 2 and 3, Plate VIII.).

Surirella gemma ought to appear well defined, and the dots intensely and remarkably black at their proper focus without any, or but little closing of the iris (Fig. 4, Plate VIII., and Fig. 2, Plate XVII., with which it should be compared).

With respect to the additional test-objects, no further information can be furnished than that afforded when speaking of them in connection with the performance of the *first-class* semi-apochromat; but it is only reasonable to expect that the increase of light inherent to the apochromat, its usually more perfect finish, its greater perfection of correction in every way, all combine to form a more perfect and in most cases a more beautifully rendered image.

Nitzschia singalensis.—This diatom is one of the severest tests for a $\frac{1}{12}$th that we know of; indeed, some think it too severe for a semi-apochromatic. It competes with amphipleura, and taxes the resources of the microscopist very severely. It is difficult to obtain mounted in realgar; and those in styrax are probably, like others in this medium in our experience, more difficult to photograph on this account. The lines in our specimen are approximately 110,000 to the inch; but they are so extremely faint and difficult to see, that the measurement must be regarded as approximate only. The object is best resolved into lines by employing oblique light, using a screen of a

maximum transmission at 4700 tenth metres, sold by Kodak, Limited, and spoken of in the Addenda, with or without the addition of No. 38, and if the student is able to resolve in green light, he has a fine objective, very fine eyesight, and an equally fine specimen. A very powerful illuminant is required. See Addenda for improvement effected by use of a nicol over the ocular.

In the photograph at × 2,600, reproduced in Fig. 3, Plate XXII., the transverse markings are shown. These lines, it will be noticed, are not nearly so pronounced or so sharply defined as in Amphipleura. When the objects are enlarged and placed side by side, as in Figs. 1 and 2, Plate XXII., this difference in appearance becomes much more accentuated, for, in the case of the latter, no amount of enlargement seems to alter their clean-cut appearance, whilst in Nitzschia an ever-increasing lateral spread shows itself. This fact should be borne in mind, as such might be put down to imperfection in the performance of the objective instead of to the peculiarity of the object. It is obvious this test-object is a very difficult one, and only appertains to the outer zone of the combination, for the strongest oblique light must be employed. With green illumination, as we have already said, the secondary markings are extremely difficult to see. They have never, to our knowledge, been broken up into dots.

Testing Semi-apochromats between N.A. ·95 and N.A. ·65.

Semi-apochromats of this numerical aperture vary in focal length between $\frac{1}{4}$ and a dry $\frac{1}{12}$ inch. We cannot help thinking there is nothing gained with these dry objectives, in having their aperture raised above ·8 at the most. This conclusion has been forced upon us after examining a very great number of objectives, in fact specimens of most of the leading opticians in the world. Whilst some computations give a satisfactory image with N.A. ·8, others require even more cutting down by the iris; but we have never yet met with one that performs to perfection at N.A. ·95, or even at ·90. We are therefore brought face to face with the belief that in the existing state of knowledge it is practically impossible for the computer to construct a dry semi-apochromatic to work really well, or even approximately

so, with any of these extremely high numerical equivalents we see advertised. In stating this the question naturally arises, of what use then is it for opticians to attempt the impossible? Is it merely to please the tyro who thinks he has much to gain by it, or is it for the more laudable object that the objective may be employed to a greater advantage with the use of oblique light?[1] The rejoinder here is, who would ever wish to push the defining and resolving power of a $\frac{1}{6}$th or any dry lens in this extreme manner when much better results could be at once obtained by using an immersion system, save perhaps under very exceptional circumstances?

In actual practice nowadays a dry $\frac{1}{12}$th or $\frac{1}{8}$th is not so commonly met with, probably because microscopists are rapidly becoming so much more familiar with immersion systems, the employment of which used to be looked upon by members of the old school with much fear and trembling. Hence what follows, although be it understood it applies quite correctly to these dry high powers in question, still is more especially directed to the examination of the $\frac{1}{6}$th and the $\frac{1}{4}$-in.; the former in particular, because so much employed by amateurs and by the medical profession as a convenient go-between the inch (or the $\frac{2}{3}$rds) and the immersion $\frac{1}{12}$th.

A. **Testing with the Abbe Plate.**—The test-plate is a most severe ordeal for any $\frac{1}{6}$th to pass through. We have several by different manufacturers in our own collection, but, as we have already stated, we have examined and tested the efforts of most firms in the world, and have yet to meet with one that yields with the full aperture (if over ·8) a really perfect image—one approaching that of a good immersion $\frac{1}{12}$th. Even when slightly reduced in aperture, these extremely high-apertured objectives do not perform to complete satisfaction, notwithstanding the most delicate adjustment for tube length, or of the correction collar—not until, in fact, their aperture is reduced to the limits we have previously mentioned.

The in-and-out focus or the use of oblique light yields the folding-over point of the spectrum in just the same manner as obtains with the $\frac{1}{12}$th, so the subject need not be further gone

[1] Upon the importance of having a large diameter to the back lens of the objective, which increases the aperture, the reader is referred to the chapter on "Numerical Aperture."

into. When, however, the diaphragm is closely shut to test the different zones in the manner already described, nearly all sixths, even of the best make, will be found to exhibit a great tendency—more especially marked in some cases than in others—to over-correction in the outer zone, and very often in the outer portion of the intermediate one also.

Some recent semi-apochromats of 4-mm. focal length certainly give a very approximately white image, such as the semi-apochromatic $\frac{1}{6}$th by Himmler, of Berlin, whilst others have a distinct tendency to that fogging of the "blacks" to which reference has already been made, and which we think so decidedly objectionable. Some of the finest specimens we have met with out of the numerous combinations examined are those by Zeiss and Watson (the Holoscopic series), Koristka, Reichert, and Leitz (the new 6A), which are all exceedingly fine lenses and difficult to disseverate in value.

When using the plate for testing correction of spherical aberration, it is necessary to be more especially careful that the best tube-length has been ascertained, or that the correction collar has been turned the exact amount to produce the finest definition. When this is accomplished it will be at once evident, the truth of what we have previously said, that with an aperture of ·95 the whole field is covered with fog and that the edges of the lines are often seen as nebulous and duplicated. If, however, the aperture be reduced to about ·8 or ·7, the best objectives at once commence to show perfect images, that combination which requires the least cutting down of the aperture of course indicating the finest computation. All other details, such as testing the zones, etc., are the same as with a $\frac{1}{12}$th immersion.

B. **Examining with Test-objects.**—Many diatoms are chosen by microscopists for this purpose, but we think the Pleurosigma angulatum comes first on the list much for the same reason (but we think with more effect) as with the immersion $\frac{1}{12}$th.

With an aperture above ·8 and ocular × 12, the black dots, if seen at all, will look exceedingly faint and greyish in most if not all cases, and they cannot be called black in appearance until the N.A. is reduced to the amount previously mentioned—namely, about ·8 or ·7, as shown in Fig. 1, Plate XIV., photographed with an excellent 4-mm. apochromatic by Koristka. If, however, the objective be a really good one, the dots will

very rapidly improve in blackness as the iris is more and more closed, the least amount required to effect this jet-black appearance indicating the finest computation.

The colour correction is very readily noticeable by looking at different parts of the specimen, especially at the edges of it, and the red becomes particularly evident to the eye when the objective is under-corrected. When the computation is arranged for the folding-over point to be at about 5500 wave-length, the purple colour is not so offensive to the eye as when the lens is under-corrected and a pronounced red is in evidence. We have said before the reason of this is not very evident, but the fact seems nevertheless true. The details concerning the performance of a $\frac{1}{6}$th with this test-object are similar to those explained when examining the $\frac{1}{12}$th immersion, to which the reader is referred for additional information. Before quitting the subject, however, a word of caution should be again given as to the use of the iris diaphragm with all dry lenses if used with a condenser of greater N.A. Under these circumstances the greatest care should be exercised that the limits of the diaphragm be exactly in accordance with the periphery of the back lens of the objective as seen by looking down the tube with the ocular removed. If this be not properly attended to and the diameter of the iris be *greater* than the back lens of the objective, light passes edgeways through the combination into the tube from which it is reflected into the ocular. By so doing it floods the specimen with light, making the definition infinitely worse than it would otherwise be. We have explained this in the article upon the use and abuse of the substage diaphragm, but we repeat the caution here, because, if unattended to, the objective may be blamed for its performance when really the fault is not due to it at all. It is worth trying the following experiment to prove what has been said is true: Having focussed a specimen, let the iris be carefully shut to the very margin (apparently) of the back lens of the objective as seen by looking down the tube with the ocular removed. When this is carefully effected, the head should be withdrawn two or three inches from the tube, and, whilst still looking into it, the iris opened, whereupon the inside of the tube will be seen flooded with light that is readily reflected into the eye of the observer. It is this light we complain of as spoiling the visual image, and

which should be carefully screened off before making a critical examination. The lesson to be learnt from this experiment is that in using a $\frac{1}{8}$th or any dry lens the aperture of the iris should never exceed the diameter of the back lens of the objective.

The Pleurosigma does not afford any idea as to the limitation of resolution possessed by any objective, and as we have never come across a test-object that is universally accepted for this purpose, we should like to bring forward the claims of the **Nitzschia scalaris** *E.* (Fig. 2, Plate XIII., taken with an exceedingly fine 4-mm. apochromatic by Zeiss). This little diatom has transverse markings easily seen, but to resolve them very distinctly into dots *with direct light* requires an aperture over ·7. *It is the difference with which such are shown* by various objectives that constitutes the superiority of one combination over another. We have used the test for years, and with a very great number of objectives, and have never found it fail. If these appear grey and only feebly defined here and there, the objective is a second-class one, whilst, if they cannot be seen at all, the lens is certainly a poor example of the optician's craft, or else has an aperture below the required amount. No attempt should be made to sharpen up the image by greatly reducing the aperture of the iris, as this spoils the validity of the test. Great care to have the best tube-length or collar adjustment is absolutely imperative, and to make the test of real value *no oblique light should be employed.* The mirror should be carefully set for direct light.[1]

The colour correction with this test-object is at once noticeable, for down the side of the valve are little spaces which always show the same colour as the lower edge of the white line of the Abbe plate when oblique light is in use. With a well-corrected lens nearly all colour disappears at the exact moment of focus, but if under-correction be present, the red colour is very plainly in evidence when the focus is but slightly changed.

[1] In making the above remarks, we are willing to admit that some microscopists, using perhaps unusually well-marked specimens, and certainly being possessed of specially acute vision, have stated that a *perfect* rendering of these dots can be seen by them with smaller aperture and less magnification. In these notes, however (concerning all test objects), we do not refer to any so happily possessed, but rather to those with average sight when employing the ordinary commercial specimen.

In some very strongly under-corrected lenses, the colour never seems to quite leave the specimen at any focus: this is a bad sign.

The Podura scale is a favourite with some microscopists. It is not used for the purpose of colour-testing, but as a test of definition, concerning which the reader should see remarks upon testing the $\frac{1}{12}$th with this specimen. If the objective be a sound one, the *constriction* around the neck of the so-called "note of exclamation" should be distinctly visible with a reduced aperture; with poor combinations, however, it is entirely lost. As nearly all these specimens were made some years ago, they mostly have thick cover-glasses, hence extra care must be exercised that the best tube-length is being employed (see page 233).

Tubercle bacilli.—Although we have never regarded any bacteria as efficient test-objects, still we might mention that these when stained with carbol-fuchsine should be easily seen with a × 12 ocular. The image ought to be entirely free from fog, hence the little red-stained rods should look *distinctly* red and sharply defined, the segregations when present being distinctly visible. With a poor objective this redness of the bacilli looks as if the organisms, after staining, had been covered over with white powder which had been subsequently blown off, leaving them slightly whitened. A faint red look then (presuming of course the specimen is a well-stained one) is to be regarded as a sign of a feeble objective. Using an F-line screen or the special pot-green glass,[1] the bacilli ought to appear as if *sharply punched out of black paper*, and there should not be the faintest possible trace of fog to be seen, save perhaps with quite the fullest aperture, but this ought to disappear directly the iris is closed to limit the N.A. to about ·8.

Additional tests may be found in the following:

Nitzschia curvula.—The dots are exceedingly faint as the diatom is excessively transparent. The secondary markings are not easy to see without oblique green light, and are always, according to our experience, difficult to photograph (Fig. 3, Plate XIII., photographed with an excellent 4-mm. apochromatic by Leitz).

Nitzschia maxima.—The dots, although usually crowded together, should be in most cases *well separated* by the objective.

[1] See "Monochromatic Light."

They should also appear *crisply* defined, especially with green oblique light (Fig. 4, Plate XIII., taken with a very fine 4-mm. apochromatic by Reichert).

The Pygidium of the Flea.—This specimen used to be a favourite test-object for objectives varying from a quarter to a twelfth of an inch. It fell out of use when large apertures came into being, because to see the specimen really well it was necessary to cut off the outer zone, as seen by looking at the back lens, the ocular being removed. Microscopists used to say "this specimen must not be flooded with light." In the present day we know the cause of the trouble arose from the corrections of the outer zone leaving so much to be desired; even in the present day, with this specimen, and especially when using a $\frac{1}{6}$th, it is better to cut off this zone, so that the testing that follows must be understood only to deal with the performance of the central and intermediate portions of the objective.

First, in selecting a specimen, care should be taken that it is mounted beneath the cover the correct way up. This is known at once by the simple fact that the long hairs coming from the wheels—to be explained hereafter—should come first into view. If this be not so, the specimen should be rejected, as all the detail of the surface of the pygidium about to be explained will be viewed through the solid portion of the object, which very materially spoils the definition.

On lowering the $\frac{1}{6}$th objective, these long hairs should come into view (see Fig. 1, Plate XVIII.), and each should appear as a delicate filament with nearly parallel sides, only tapering to a point not far from their ends, and then somewhat suddenly. Lowering further, the hairs forming the outer covering of the pygidium, and constituting part of it, crowd into evidence, as shown in Fig. 1, Plate XIX., and more amplified in Fig. 2 of the same plate. They should appear *entirely* free from fog, sharply and very clearly defined. They are not "spines," but appear, as Dallinger writes, as "lambent bulbous hairs." They are interwoven together so much that they are not easy to disassociate one from the other; besides which, there seems to be more than one layer. The point for the student to notice is the quality of the seeing. It should be clean, sharply cut, and entirely free from all fog (see Fig. 1, Plate XIX.). The iris should only require to be slightly closed.

Lowering the objective (the $\frac{1}{6}$th) still more, the so-called "wheel discs" present themselves to view, and note should be taken that some may differ in their number of spokes (see Fig. 3, Plate XX., and more magnified still in Fig. 3, Plate XVIII.). The cuneiform, or Maltese-cross appearance of these spokes is very striking, the central dot being the hair that issues from each wheel. These wheels should appear with extreme sharpness, not a trace of fog being present, although they may not be all in focus at the same time. When focussed sharply, and without touching the fine adjustment, the substage diaphragm should be opened as wide as possible (anyhow, to entirely expose the area of the back lens of the objective), and a careful inspection of the image made. If the $\frac{1}{6}$th in use be really a fine one, notwithstanding the amount of fog introduced, the discs should be still *distinctly visible* and the peculiar shape of the spokes *remain the same* (see Fig. 2, Plate XVIII.). Certain objectives will now break up in their defining powers, especially those with poorly corrected outer zones, for some have been known to actually show the cuneiform spokes as "dots," and so clearly indeed, that observers in the past have recorded this dot appearance as the correct one, not even having seen the Maltese-cross effects!

It will be gathered from what has been said that the pygidium wants care in using as a test-object. It can be employed just as well with a $\frac{1}{12}$th, for the great point about the observations is, the absence of fog and the smallness of the amount of closing required of the substage diaphragm to obtain these fine effects.

Stauroneis phœnicenteron.—This diatom is a good test-object for a third, with a numerical aperture of ·65 or thereabouts. See Fig. 3, Plate XIV. Specimens mounted in styrax usually yield good results and are easily obtainable. The dots are about $\frac{1}{85500}$ of an inch apart, and resolution at the aperture in question ought not theoretically to be difficult; but we have found that to see them clearly and distinctly is not quite so easy a matter. A weak objective is prone to furnish a good deal of fog: it may arise from the high eye-piecing that is necessary. If, however, a good semi-apochromatic be employed, or a well-corrected apochromatic, the dots should be distinctly in evidence, especially when green light is in use. With the

little arc-light and the blue screen, with or without No. 38, the apochromatic should furnish a really fine image, and when green light is substituted with the semi-apochromatic, the rendering ought not to be much if at all inferior. In all cases, attention to the tube-length is demanded if the finest black dot effects are required. The striæ, according to Van Heurck, are about 14 to the c.d.m. (see Index).

Testing Apochromats from N.A. ·95 to ·65.

With respect to the testing of the apochromatic objectives of the apertures above mentioned, ranging in focal length from $\frac{1}{6}$ to $\frac{1}{2}$ in., there is little further to be said beyond that stated when dealing with semi-apochromats of the same numerical equivalent employed upon the plate; but the leading feature of difference is the purity of the image and the freedom from colour within and without the focus.[1] The same precautions, however, as to using oculars properly compensated hold with these objectives as with the 2-mm., but of course the deteriorating effect of inferior eyepieces is not so pronounced because the image is not so magnified. The reader is referred for further information to what has been said when this higher power objective was under a similar examination.

Amphipleura pellucida.—As a test object to show the lines with or without a trace of the dots, this is the most difficult test object for an apochromatic sixth in existence, as the outer zone must be of exceptional excellence, extreme oblique being required. It is still more difficult to photograph. An attempt is shown in Fig. 4, Plate XV.

The lines in Nitzschia scalaris (Fig. 2, Plate XIII.) can rarely be perfectly resolved into discrete dots if the objective has an N.A. below ·70; but the reader is referred to the footnote on page 398. It should be recollected, however, the absence of the

[1] The apochromatic of a $\frac{1}{4}$-in. focal length by Zeiss, only made for the long-tube, is an objective not nearly so well known as it should be, for it is truly a magnificent combination. A fine test-object is the little diatom called Van Heurckia Louisiana. Transverse lines exceedingly close together can be seen on either side of the median raphé; but a very fine combination shows portions of these lines resolved into dots. It is a somewhat difficult subject to photograph. Fig. 1, Plate XV., was taken with the objective in question.

secondary spectrum assists so much in the apparent definition of this object that the observer is often apt to think the actual performance of the apochromatic objective *per se* is very much finer than its rival. To test this properly, let him place a green film screen, or a piece of the green glass previously mentioned, between the illuminant and the mirror and then compare the performance of a really fine semi-apochromat with the entire apochromat used under similar circumstances; he may then be surprised at the small amount of difference. Of course this is really not the point; the question is, how do they differ without using any screen at all?

Navicula cuspida.—This diatom, shown in Fig. 1, Plate XXI., has been suggested as a test-object—perhaps more especially for an apochromatic *sixth* objective. It requires the most patient study. The student is recommended to purchase a specimen put up in realgar—anyhow, to commence with—for those mounted in styrax, in our experience, do not show the markings so well. Using one mounted as we suggest, and employing a $\frac{1}{6}$th apochromatic, a solid cone of white light of ordinary intensity, a ×6 ocular, a full aperture, and with a 1·30 oil-condenser oiled to the slip (as this seems to give better results than a dry condenser), the transverse lines are easily seen, being recognisable up and down the entire valve, which for convenience of description is placed vertically in the field of view. With a ×12 ocular and very careful focussing, a faint row of very small black dots may now just be caught and held by an observer if he has a very sharp eye and a fine lens. Upon using a ×18 eye-piece these dots are more easily seen, and if green illumination be adopted, they certainly appear plainer still, especially if the iris diaphragm be closed a trifle. No semi-apochromatic with which we are acquainted—and we have tried several—will offer more than a trace of these secondaries. Changing now the green screen for a blue-violet (maximum transmission at 4700 tenth metres), and with it the No. 38 screen, in company with the little arc lamp mentioned on page 167, the little dots should become very plainly visible, and that indeed without any straining of the eyes at all. On altering the focus, a surprise awaits the microscopist, for white dots ought now to come into view, being fairly well seen; but they appear due to the resolution of the *spaces between the*

transverse lines, and not to the breaking up of the lines themselves (see Fig. 1, Plate XXI.). A first-class semi-apochromatic may just reveal a trace of these, but, in our experience, it is but a trace. Making the blue-violet illumination oblique—the circular opening of the substage diaphragm being equal in diameter to about a third of the diameter of the back lens of the objective, as seen when looking down the tube of the microscope, the ocular being removed—a position will soon be found for this opening to be placed, which ought to furnish a very fine image of the white dots, all arranged in clear and distinct rows, separated by discreet intervals (see Fig. 1, Plate XXI.). These appearances, it may be remarked, ought to be fairly well shown by a first-class semi-apochromatic, especially if the iris be closed a very small amount. The student must now be prepared for what to us is an unusual appearance. No change of focus seems able to convert these white dots into black ones as mostly obtains with diatom structure; but careful focussing reveals the fact that while the white dots are sharply in evidence, certain black patches can be seen arranged somewhere around them, their exact position varying according to the arrangement of the oblique light. They are not circumscribed like dots, but are best described by the word "patches" of blackness. This is more apparent if the magnification be increased by eye-piecing. What, however, is so remarkable, is that their position seems capable of being varied according as the azimuth of the oblique pencil is changed; and that they appear at the same focus as the white dots. These patches are strangely like shadows, but this is believed to be impossible when the objects which are supposed to cause them are commensurate with the length of a single wave-length of light, or thereabouts. To what they are due then, it is difficult to say, but it may be surmised they are caused by interference phenomena of some kind, complicated very possibly with some residuals of aberration in the combination in use. To make our meaning more intelligible, we have enlarged Fig. 1, Plate XXI., and reproduced the positive taken therefrom in Plate XXIII. This shows the white dots (looking like little mountains) and the dark patches, of which we have spoken, both in focus at the same time.

Specimens in styrax exhibit these appearances quite efficiently, though they are better rendered if they are mounted in realgar.

The photographs, however, were taken from a valve in the former medium.

Testing Objectives from ·65 to ·2. Semi-apochromats and Apochromats

Objectives having this range of N.A. usually possess a focal length varying from one-third of an inch, or a half-inch, to an inch in length. Some English and Continental manufacturers make shorter focal lengths of this low resolving power, but they are especially constructed for the purpose of giving a high magnification with great depth of focus.[1] Such must be judged by their aperture and not by their focal length.

The testing of the ⅓rd—which has usually an aperture varying from N.A. ·65 to N.A. ·40—may be carried out with the Abbe plate in the fashion already described, for the point of folding over of the spectrum; but to discern the class of definition afforded, and the resolving power, it is better to use a test-object. To find a suitable one, however, is no small difficulty. We have always employed the Nitzschia scalaris, *using oblique illumination*, care being exercised that the incident light shall fall parallel with the lines and not at right angles to them. With an apochromat of ·65, the lines should be distinctly visible (see Fig. 2, Plate XIV.); but the test is indeed a severe one for a semi-apochromat, unless it be of exceptional excellence. In either case the correct length of the draw-tube must be *very* eagerly sought, for the slightest error in this respect is sufficient to nullify the test. With apertures of less than N.A. ·60 it seems impossible to use this diatom in the manner explained; hence, with objectives of lower numerical aperture, we are fond of employing the Navicula major. A good lens of N.A. ·40, well corrected and indeed in some cases one of ·30 aperture, should show with a × 12 ocular the tongue-like transverse striæ, whereas one of second quality will only faintly indicate their presence, and a really poor specimen of optical construction will fail to furnish any indication at all of their existence. It should be borne in mind, any objective from ·65 to ·30 should

[1] These are especially of service for studying objects that are motile, as the specimens do not go out of focus nearly so easily. See article, "Depth of Focus."

easily bear a × 12, or even, with a suitable object, a × 18 ocular, without producing a "rotten" image.

Another test for low apertures is the Proboscis of the Blow-fly (Fig. 1, Plate XVI.). Although this is an old-fashioned one, still it holds its own with many, if not all opticians. The image of the suctorial tubes should appear crisp and *very cleanly cut*, whilst the exceedingly small hairs, especially those about the outlying borders of the little organism, should be *black* and very sharply defined with critical light. *There should be an entire absence of fog over the whole proboscis* after adjusting the iris to exactly the dimensions of the back lens of the objective as seen by looking down the tube; and the amount of additional closing of the iris necessary to clear this off is a guide to the perfection of the design and workmanship of the lens under examination; but nearly all combinations, however, require a *small* amount of closing of the iris before producing their best effect. A flat field is, of course, with low powers especially, a great desideratum, and the loss of it is perhaps more particularly unpleasant with low magnifications than with high ones. A great improvement, however, in the modern low powers has been effected in this respect, especially since the advent of the Jena glass, which leaves in the present day but little to complain of in any way (see Frontispiece). On this account it is almost impossible to say one lens is better or worse than another, for the product of nearly all opticians is so remarkably good.

The inch combination that has the high numerical aperture of ·3 is not so commonly met with, save in the case of some apochromats. To select diatom tests just suited to distinguish a combination of ·3 from that of less aperture, such as ·2, has not been easy. The following, however, may be of interest to those who are fond of testing lenses; but it should be remarked in passing, that as the tests are severe, very careful adjustment of the light and very careful focussing are necessary.

The first is Actino-ptychus Boliviencis, a diatom easy to obtain. A combination having a numerical aperture of ·3 should neatly pick out the points in three of the sections (the remaining three being necessarily out of focus), but care is needed to obtain a good image. It is just possible to see the dots with an aperture of ·24 (·17, in our experience, being useless), but the higher numerical equivalent is required to show

them as seen in the photograph, Fig. 3, Plate XV. A strong green light is a great help, but the 4700 screen, with or without film 38 and the small arc light described on page 167, furnish definition of a far superior kind.

The second diatom—Hemidiscus cuneiformis—which is also readily obtainable, is much more difficult. It has seemed impossible to obtain a resolution into dots, similar to that shown in Fig. 2, Plate XVI., with less aperture than ·3, a faint suspicion only being seen with N.A. ·24. It seems imperative also to use slightly oblique green light, and it should be borne in mind that oblique light is best obtained with such low powers as an inch by a suitable shifting of the mirror, rather than by employing the usual substage arrangements for higher powers. The blue light arrangement above mentioned renders the seeing far cleaner and more distinct.

The third diatom is the well-known Navicula Smithii. It is best to have a few specimens to select from, as this test is one of the most severe we know for the aperture in question. Further, it is better to obtain the diatom mounted in styrax. Much care and time may be required in arranging the light to resolve *any* of the transverse lines, and it has seemed impossible to do so up and down the entire valve at one and the same focus. In the photograph, Fig. 3, Plate XVI., several dots can be seen in each specimen (especially if a hand magnifier be used), but the objective must be a well-corrected one to produce as good results. Oblique blue light renders the seeing somewhat easier than when oblique green illumination is employed; but in our experience the specimen is a difficult one to negotiate.

It is needless to remark that apochromats of N.A. ·3 *should* furnish no colour with the image of *any object*; but it must be stated at the same time that the Abbe plate always seems to show with this lens a certain amount of colour, usually reddish, left outstanding which the computers declare is practically impossible to remove even with the best of design and workmanship; but this might be of a tertiary nature; a holoscopic adjusting ocular will sometimes remove it in part.

With the combinations under consideration, it is necessary to use condensers that give an evenly illuminated field. This implies their focal length must be long and their aplanatism very perfect; whilst at the same time their numerical aperture

must be equal to that of the objective. To accomplish this latter result, they must necessarily be of large dimensions; but as it is of no consequence for them to be so accurately centred as obtains when illuminators are used with the high powers, more room can be granted them, for the centring apparatus is no longer required. Hence the optical part only of these large condensers is usually made simply to drop into the sleeve of the substage. Zeiss's loups *are very excellent for this purpose*, and so are many condensers by several opticians, although we admit to have a great liking for the specially low-power constructions sold by Watson and Sons, under the name of Holoscopic condensers—their performance leaves nothing to be desired.

Testing Objectives below N.A. ·3

These are usually of a focal length varying from 1 inch to 3 or even 4 inches. There seems no distinctive difference in the performance of apochromats and semi-apochromats from good achromats with this long focal length, save when used for the purposes of photography. Their construction is not regarded as a very difficult feat, and most opticians sell excellent examples of manufacture. The absence of fog with the full aperture when using the proboscis seems a leading test, and how much the iris has to be shut to effect a sharp and clean image serves to distinguish the good from the bad specimens of workmanship and design. A suitable condenser of similar N.A. should be employed, or the field of view will suffer in uniform illumination and perhaps in definition. A low-power "loup" or a Holoscopic condenser are useful.

Apochromat *versus* Semi-apochromat. Which shall be Purchased?

This question, often asked by the commencing student, no matter in what department of Microscopy he has cast his lot, requires a careful reply.

The only difference, optically speaking, between the two types of objective referred to should be the entire absence of the secondary spectrum in the apochromat, whilst it is left outstanding in the semi-apochromat. To the reader who is not optically inclined, and who is practical rather than theoretical, this explanation we have given affords no material comfort; in

other words, he is as wise as he was before. What he wants to know is the *practical difference* in daily use between the rival systems.

Let the reader so inclined take a diatom such as a Navicula lyra and place it on the stage, using a semi-apochromatic $\frac{1}{12}$th. Whilst he is bringing to a focus the edge of the boat-shaped valve, he will be sure to notice that there are colour phenomena visible, apple-green before he arrives at the focus, and purple should he go too far beyond it. With closer attention still he will soon observe that the little dots of the diatom, small as they are, show the same colours as the thick-edged border when the objective is raised or lowered. These are the colours of the secondary spectrum spoken of a few lines back.[1] If now the lens be changed for an apochromat of the same focal length, he will not fail to notice the colours so pronounced before, are now entirely absent, for the specimen is an excellent one to show chromatic correction. Then, too, he cannot fail to be conscious of the greater brightness of the image which arises from the fact that a greater part of the total light of the spectrum is concentrated in it. It is quite plain then that the apochromat, when used with diatoms in the manner explained, holds its head far above its rival. But experience has taught the philosopher "never to despair," for those who keep us moving ahead by their experimental researches and their theoretical considerations (to whom we all owe our gratitude) not so long ago pointed out (i) that as green light would furnish greater resolution than the rays of maximum effect in white light, because of its shorter wave-length, and (ii) because it would cut off the secondary spectrum with all semi-apochromats, and lastly (iii) because the colour in question was very closely that of the minimum focus of all these modern objectives—it should, if selectively employed,

[1] It should be recollected by the student that these colours *should* be seen: if absent, unless the combination be an apochromat, it is quite likely that such absence of colour is brought about by the computer leaving residuals in all the zones, even with the preferred colour, which means that the definition really is impaired by a kind of cloud or fog, which prevents the "blacks" really looking as black as they should. Even with modern lenses this "trick" is occasionally found to be present, and the student may feel pleased at the absence of colour effect, quite forgetting that such is produced at the expense of the finest definition and crispness of the image (see page 364).

improve the image with this type of lens-construction to a very great extent. This has been found to be actually the case, and in the most marked manner; so much so that it is not easy to distinguish the two results—that produced by the apochromatic and the semi-apochromatic—*when both are used with green screens*, provided the observer, to avoid being prejudiced, has no clue as to which objective is in use.

Unfortunately at times, the green filter is not suitable for the object—perhaps that itself is stained with methyl green—and so another screen has to be used of a different tint to produce contrast (if indeed a suitable one can be found). Here the semi-apochromat may fail to satisfy, because we have already shown that it does not perform so well when used with a screen of a different colour from that for which the lens is corrected; which is, after all, but a natural consequence of its manufacture and construction. Here then the apochromat again resumes its superiority, for its performance is just as good with all colours if the combination in use be a really good one.[1] If, however, bacteria are employed as test-objects, seeing they are usually red-stained, and as, moreover, they do not possess in ordinary cases any refinement of detail, the difference in the performance (visually) between a first-rate specimen of either type is not strikingly in evidence. There are a few cases, however, when appeal may have to be made to the apochromat, but for general purposes its cheaper rival will answer all the requirements demanded. The same remarks apply to the lower powers: when the finest definition possible is desired *entirely free from colour*, the apochromat must be the selected lens.

But the economical side of the question has to be discussed. The semi-apochromat, owing to its simpler construction, can be purchased at a price very considerably less than that justly demanded for the apochromatic with its eight or more com-

[1] The three colours are brought to a focus in these remarkable lenses in such a manner and with such refinement that the actual focal differences of the various positions of the spectrum are reduced, we believe, to something like one-eighth of their original magnitude, which means that practically they are eliminated; besides which the corrections are complete for all the zones of the objective instead of for one only, as obtains with the older type of lens. For further interesting details the reader is referred to the chapter devoted to the "Testing of Apochromatic 2 mm. Objectives," and the Addenda.

ponent lenses. Hence in everyday use, when perhaps the very highest attainable degree of optical excellence is not actually required, where differences of form and shape are perhaps more especially sought after rather than the ultimate resolution of the minutest details—for such purposes then the semi-apochromat answers perfectly well if it be really well made. There is, too, the satisfaction when using these cheaper combinations of knowing, that if in the hurry of work, when the microscope is used as a tool rather than as a thing of joy, should any accident occur to the front lens of a semi-apochromat it can be replaced at much less cost than would be the case with its more expensive rival. Yet on the other hand, no laboratory or battery of an amateur, or of a research student, can be said to be *complete* without apochromats; but what we wish to be understood is that for ordinary visual work the semi-apochromat, if it be really a good one, is of sufficient quality for all general purposes, but that a first-class apochromat should be within call as a court of appeal.

With respect to their use in photomicrography, however, all is different. We do not deny that some semi-apochromats, especially low powers, when used in conjunction with *suitable* screens, produce excellent pictures—as, for example, the lens with which the frontispiece was taken; but, speaking in general, with high powers this is not the case, and those who desire to obtain the *very finest results possible*, by the aid of photography, should certainly obtain the service of the apochromat rather than that of its cheaper rival.

Low-power Magnifying Combinations not used on the Microscope

A demand has arisen of late for photographs to be taken of whole insects, or of some other occupant of the animal kingdom of small size, such as fleas or lice, at magnifications varying from a few to several diameters. Sometimes, in addition, principally for identification purposes, a request is made that parts of the same, such as the entire head and thorax of an insect, or the wings and body of a mosquito, shall be furnished at a greater amplification still. With an ordinary microscope and microscopical objective this has been found in many instances an impossibility, partly owing to the limit of view imposed by the

ocular, or by the diameter of the draw-tube on the one hand, and on the other by the fact that no ordinary objective constructed for the microscope gave a sufficiently large and flat field—that is, one furnishing crisp definition simultaneously at the centre as well as at the edges of the photograph of so large an object. Giving the microscope a much shorter and wider tube, or by doing away with the microscope altogether and using an ordinary camera provided with a focussing jacket, assisted in one direction it is true; but even then it was found necessary, for reasons aforesaid, to design a new type of combination to meet satisfactorily the requirements of the case. Seeing that these lenses are used for magnifying purposes—for they are really microscopes of low magnifying power—and that but little has been said or written about them, it was thought the following few remarks upon the subject, accompanied by illustrations, would not be out of place in this new edition of "Microscopy."

There are five points to be considered in relation to this subject. (i) It is necessary to have four combinations of different focal length, a one, two, three, and a four-inch, this being required owing to the different sizes of objects to be photographed and to the varying magnifications demanded, and also to avoid in all instances the employment of a camera extension exceeding three feet, longer than which it was found inconvenient to use single-handed.

(ii) The combinations must be of large aperture, F/4 to F/6 being that mostly selected. This will be understood as necessary when the reader is reminded that the N.A. of a photographic lens is obtained by dividing the F ratio into ·5 (see page 145). Hence the N.A. of a combination working at F/4 is ·125, whereas that of another working at, say, F/8, has but half the resolving power. It is true that the details required to be photographed are not, as a rule, so exceedingly small as to require a great N.A., but still the more the N.A. that can be employed, the better the final result.

(iii) The field must be exceptionally flat, so that the definition at the margins of the negative, even at its full aperture, shall be sensibly equal to that at the centre, the iris diaphragm being only employed (so far as possible) to give the required depth of focus. This severe demand is imperative, as attention has sometimes to be called to the special formation of perhaps an

outstretched foot of an insect, the extreme end of a proboscis of some fly, or the fine hairs on the edges of the wings of, say, a mosquito (which may materially assist in deciding the variety to which it belongs), at the same time as attention is being called to portions of the body.

(iv) An astigmatic condition of correction must be in evidence, otherwise markings that happen to be at right angles to each other at the margins of the field, will not be in focus simultaneously, and details looked for may not be found, being obliterated from this cause.

(v) The corrections for colour should be almost of an apochromatic order, anyhow so perfect that, if a focus be obtained in white light, but little, if any, sensible change is produced by the introduction of a coloured screen for contrast purposes.

In comparing and testing lenses of this type, which are placed on an ordinary camera provided with a focussing jacket, certain precautions have to be observed. Putting aside the providing of a suitable illumination and the equal distribution of the light over the entire portion to be photographed—which belongs to the realms of Photomicrography—when combinations are to be compared one with another, it is imperative they should be of the same focal length. Variations in the depth of focus—which results from the difference in area of the actual lens surface of glass exposed (compare, for example, the difference in area of F/16 of a three-inch with F/16 of a one-and-a-half-inch), is quite sufficient to spoil the final effect, that is, if the operator be not conversant with the true knowledge of the cause, and so is prepared to take proper precautions. Seeing that *the smaller the amount of the diameter of the glass exposed, the greater the depth of focus*, we may add, as a corollary, that the shortest focal length that can be used, should always be employed, because of the greater depth obtained, and consequently of the less shutting down of the iris diaphragm required. Before proceeding further, we should mention that two negatives have been made to illustrate this point, each at F/16, the first with a three-and-a-half-inch focal length objective, and the other with that of an inch-and-a-half (Figs. 1 and 2, Plate XX.). Both lenses were focussed on the antennæ of the blow-fly—the two sausage-like appendages hanging side by side in the centre of the pictures. The diaphragm was then closed to F/16 in each case and the

negatives made. As the specimen is mounted without pressure, it affords a good opportunity for comparing the relative amounts of depth of focus provided by each combination. Let the reader compare, for example, the appearances of the compound eyes and the fine hairs attached thereto. The shorter focal length (Fig. 2) distinctly shows a great many of the facets and the hairs fairly sharp, whereas the three-and-a-half-inch (Fig. 1) hardly furnishes anything more than a faint and shadowy existence of the facets, whilst the hairs are distinctly out of focus. It is true it may be said that if the iris of the longer-focus combination was shut down so that the diameter of the lens surface exposed should be similar in extent to that of the 1·5-inch (putting aside, for the moment, the reducing effect upon the value in numerical aperture), the depth of focus would be the same, but when comparing lenses for efficiency, it is not scientific to compare an F/16 of one combination with perhaps F/22 of another. It should be remarked in passing, that it is not always possible to reduce the aperture of these lenses to F/16, for in some instances, owing to the loss of numerical aperture, the details will appear quite fuzzy and ill-defined, but it must be understood that these remarks apply just as truly to any other apertures that may be compared. A remark was made previously about the utility of having different focal lengths to use, so as to save inconveniently long cameras; it may be mentioned here as illustrative of that remark, that the camera length for the 3·5-inch lens (Fig. 1), in taking the photograph above given, reached to five feet, a most inconvenient camera draw to manipulate.

The first lens to fulfil the requirements mentioned was the "Planar," by Carl Zeiss. It met with so much success and was in such great demand, that opticians, both English and foreign, soon computed competing combinations—the "Holos" by Watson & Sons, the "Micro-Summar" by Leitz, the "Polar" by Reichert, and the "Beck-Steinheil" by Messrs. Beck, not to mention a few others, all being combinations of large working aperture and of great excellence.

Illustrations of the work and performance of most of these lenses are given in the five Plates, XXIV. to XXVIII. inclusive. The examination of these photographs will show the realisation of the requirements previously enumerated, and it is of interest

to carefully note the excellence of the focus over the large areas involved. The names of the lenses used have been purposely omitted, as it was thought that perhaps any indifferent results, whether they arose from faulty work of the author, or some slight imperfection in reproduction, might prejudice the reader.

In testing these lenses, if no photographs can be conveniently taken at the moment, the images to be compared can each be examined with a hand magnifier (carefully adjusted), using a plain glass on the camera in the place of the usual ground glass. It is needless to point out that the specimen must be set at exactly right angles to the axis of the lens, for the slightest error in this respect will cause one side of the image (and consequently the photograph) to be distinctly out of focus. To obtain the best results, means must be provided to slightly move the object under examination, so as to approximate to the lens either edge, whether at the top, bottom, or either side, for the purpose of correcting any slight differences of level in the mounting of the specimen, or even, perhaps, in the object itself.

To recapitulate then, the leading rules in testing or using these lenses is first, to select one with as short a focal length as consistent with the demands of the case, because of the gain thereby in depth of focus without loss of numerical aperture, and, secondly, to employ the largest aperture of the iris that is consistent with the required depth of focus, because of the *increase* of numerical aperture thereby obtained. See Plates XXIV. to XXVIII. inclusive.

CHAPTER XV

THE UNDULATORY THEORY OF LIGHT

WHENEVER we try to get the utmost resolution out of any optical instrument by increasing the magnifying power beyond a certain moderate limit we are confronted with facts which run counter to the theories of geometrical optics, and which can only be accounted for by taking into consideration the undulatory nature of light—*i.e.* by rejecting the fiction of geometrical optics according to which light consists of infinitely thin rays which can be united in points, and by applying instead the principle of interference to these problems.

A clear understanding of the elements of this undulatory theory of light, now universally accepted, is therefore essential to the student of microscopy; fortunately this is not so difficult to acquire as many people imagine.

The principal facts which have rendered rival theories impossible, and have given the victory to the undulatory one, are the numerous phenomena of interference which can only be explained by attributing to light a periodic or vibratory character, and the further phenomena of polarisation which force us to the additional conclusion that the vibrations are transverse ones—*i.e.* at right angles to the line of progression. Vibrations call for a medium in which they take place and by which they are transmitted; in the case of light this medium is the all-pervading "ether" or "æther," hypothetical in so far as it has never been isolated in the chemist's sense of the word, nor has it had any material properties assigned to it, but nevertheless an unquestionable physical reality in the sense in which it enters into physical theories.

We will here adopt the assumption of the early investigators of the undulatory theory as to the constitution of the ether,[1] viz.

[1] In scientific language this means that we are adopting the theory according to which the ether is an incompressible elastic solid.

that it may be treated as consisting of minute particles elastically connected with one another in such a way that when any particle is displaced from its position of rest it communicates the disturbance to those surrounding it, these in turn to their neighbours, and so on. As obviously a neighbouring particle cannot move until the preceding one has begun to do so, time must be consumed in the process of propagation, hence the finite though exceedingly great velocity of light. This simple conception is sufficient for our purpose, and is undoubtedly easier to grasp and to follow than the more abstract—if more philosophical—assumptions evolved since those days.

We therefore assume that the light emanating from a self-luminous body is due to extremely rapid vibrations of the smallest particles (molecules) of which the body is composed. These vibrations are communicated to the surrounding ether, and are propagated in it in all directions in the manner just described.

Referring to Fig. 208, where P is supposed to be a luminous

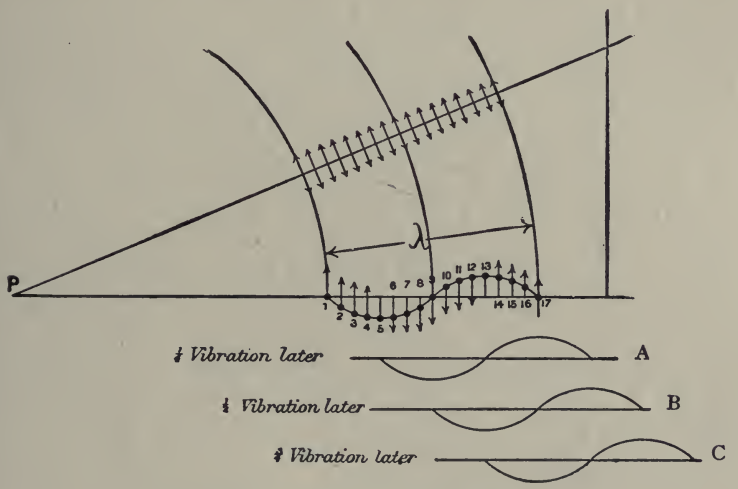


Fig. 208.—Nature of Light-vibrations.

point vibrating in the plane of the paper, this vibration will be communicated to the surrounding ether from particle to particle; a rather difficult investigation shows that the result at some little distance from P is that the ether particles are

set vibrating in a direction at right angles to a straight line connecting them with the luminous point and with an amplitude or length of path depending on the brightness of the point P and on the distance from it, in the manner shown in the upper part of the figure; that is to say each ether particle remains for ever close to its position of rest, vibrating across or around it with extreme rapidity, but in a path of most minute length. There is thus neither ether nor anything else transferred from a luminous point to the objects illuminated by it.

We will now study the movement a little more in detail as shown in the lower part of the figure, where the black dots signify a succession of ether particles whose position of rest would be on the radial line from P. Supposing that at a certain moment the particle 1 is just crossing its position of rest, moving *upwards*, then particle 2 will be slightly lagging behind, thus approaching the radial line and so on until we find a particle 5 at the reversing point of motion, or elongation. The particles still farther from P have not reached the lower elongation, and take the successive positions indicated until we come to particle 9, which is just crossing the radial line, moving *downwards*. The succeeding particles are found in various positions above the radial line—particle 13 being at upper elongation—until we finally come to particle 17, which is in the same *phase* of the vibratory movement as the first particle—*i.e.* crossing the radial line in an upward direction. Evidently particles still further to the right would be found in positions exactly corresponding to those we have just studied, and would repeat the curve to which the first series of vibrating particles is seen to conform. The distance from particle 1 to particle 17, or generally from any one particle to the nearest one simultaneously in the same phase of movement, is called the wave-length of the light from P, and is usually designated by the Greek letter λ. Once we grasp this peculiar movement we can easily see that, say, $\frac{1}{4}$ vibration after the time when the position of the particles is as in Fig. 208, particle 1 would have reached its upper elongation and every other particle would also be in a correspondingly advanced stage of its movement. The result, as shown in Fig. 208 A, would be that the curve connecting the successive ether particles has advanced $\frac{1}{4}$ wave-length; similarly $\frac{1}{2}$ vibration later we shall have the appearance shown in

Fig. 208 B, the wave curve having advanced ½ length, and so forth; the result being that the curve connecting successive ether particles advances one wave-length for every complete vibration, and it is to be noted that this curve—a mathematical line only existing in the imagination—alone can be looked upon as moving in the direction away from a luminous point at the velocity of light. One other point we must notice: The light from P proceeds simultaneously in *all* directions, hence at any given moment the ether particles at some given distance, such as that from P to 1, will all be in the same stage of vibration; in the case of our diagram they will all be crossing their position of rest, travelling upwards; now points equidistant from a given point lie on a sphere of which the given point is the centre, hence we conclude that we can describe an unlimited number of spheres round P as centre, all of which have the property that all the ether particles lying on any one of them are always in a similar state of vibration—*i.e.* all moving upwards at one instant, all at upper elongation a little later, all moving downwards yet later, and all reaching lower elongation at a still later moment. Surfaces thus characterised are called *wave surfaces* or *wave fronts*; near a source of light, and as long as the light is proceeding in one uniform medium they are spherical, as we have just seen. At a *great* distance from their source their curvature becomes so slight that we can regard small parts of them, such as can enter a small object-glass, as *plane*, and we then speak of *plane waves*; finally, when undisturbed spherical or plane waves enter any kind of lens system they suffer refraction; in the ideal case they are turned into truly spherical waves converging towards the conjugate focus of the lens system; in reality all lens systems are more or less afflicted with "aberrations," which, from our present point of view, means that they turn the arriving light-waves into refracted waves which are more or less *distorted* from the ideal spherical form.

Perhaps the most remarkable feature of light-waves is their extreme rapidity of vibration, which ranges from about 400 billions[1] of complete vibrations per second for red light to nearly twice as many for violet light, and we must here note at once that this number of vibrations per second is the only characteristic

[1] The billion is here used as signifying a million millions.

of light which is unchangeably connected with its colour as seen by the human eyes. In empty space light travels at the rate of 300,000 kilometres per second; the above number of vibrations per second is therefore contained in this distance, and if we divide 300,000 kilometres, or more conveniently the equivalent 300,000,000,000,000 microns by 400 and 800 billions of vibrations respectively, we find the wave-length of red light as about three-quarters of a micron, that of violet light as about three-eighths of a micron.[1]

In denser media, such as water or glass, light travels more slowly, and moreover at somewhat different speeds for different colours, and as the number of vibrations is an unchangeable quantity for any one colour, there results a shortening of the wave-lengths in such media. Hence the statement of the wave-length of any particular kind of light should be accompanied by a statement of the medium in which it is measured; it is, however, always assumed that wave-length *in vacuo* is meant unless there is a statement to the contrary.

Besides their amplitude and their rate of vibration, light-waves have one more characteristic. We have hitherto assumed that our luminous point P was vibrating in an up-and-down direction; but there is no necessity why it should be vibrating in that particular direction; indeed, if we imagine ourselves looking at the luminous point, it is just as probable that it may be vibrating in a horizontal or oblique direction as in the vertical one. This distinction as to the *azimuth* in which the vibrations take place is of secondary importance in the consideration of interference phenomena; it becomes important, however, when we use the polariscope; it is indeed this azimuth of the vibrations which determines the state of polarisation of light. In some of the polariscopic experiments the vibrations assume a yet different form; *i.e.* the ether particles gyrate round their position of rest in elliptical or circular paths, whence arise the terms elliptical and circular polarisation.

In ordinary light the vibrations take place indiscriminately in all possible azimuths, and as every useful source of light contains an enormous number of vibrating molecules, all of which are vibrating more or less independently of one another, the light from such a source contains vibrations in all possible

[1] Say $\frac{1}{25000}$ in. and $\frac{1}{50000}$ in. respectively.

azimuths simultaneously, and is in consequence free from any trace of polarisation.

We will now proceed to the consideration of those cases which are of especial importance in the theory of optical instruments, viz. cases where a number of vibrations act simultaneously. Let us assume that light from a point P_1 at a given instant would, if acting alone, produce the wave I, Fig. 209A, that light from another point P_2 would at the same instant, if acting alone, produce the wave II; what is the result if both act together? The answer is supplied by the fundamental physical principle of *superposition*, according to which the position of each ether particle is such as would result if it was first displaced according to wave I and immediately after according to the displacement of wave II. All we have to do therefore to get the combined

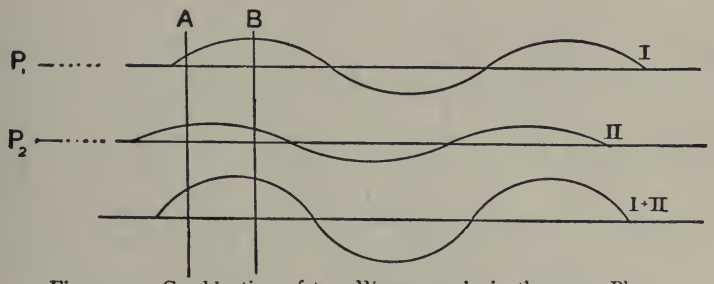


Fig. 209A.—Combination of two Waves nearly in the same Phase.

effect is to take a number of points like A and B, and superpose or add the displacements in that position. Thus at A both waves gave upward displacements; the combined wave accordingly has an upward displacement equal to the sum of those of the components, the result being a new wave of much greater amplitude than that of either of the combining waves.

The two waves combined in Fig. 209A were of nearly the same phase; a very different result is obtained if the phases of the combining waves are more or less opposed, as in Fig. 209B. Here we at once notice that nearly everywhere the two separate waves are of opposite tendency, hence the combination now gives displacements approximately equal to the difference of the combining displacements and the resulting combined wave has a less amplitude than the stronger of the combining waves.

Having learnt how to combine two waves, we can immediately proceed to combine any given number by combining them first in pairs, then the resultants again in pairs, and so on until the total result is obtained ; or we may combine the displacements

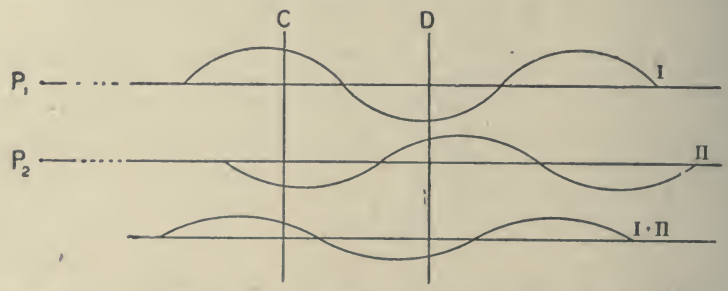


Fig. 209B.—Combination of two Waves opposed to each other in Phases.

of all the separate waves in one operation and obtain the same result.

Before taking an example of such a problem, we must however discuss the result of the co-operation of two or more waves more closely ; for whilst what we have learnt is strictly true under all conditions, no matter how many waves are acting at the same time, or what relation as to wave-length and state of polarisation they may be in, the result *as far as human eyes can see it* or photographic plates can record it, is subject to very decided differences. With the aid of Figs. 209A and 209B we found that the result of the combination depends chiefly on the phase-relation between the combining waves ; if both are in the same phase a wave of great amplitude results, if the phases are opposed to each other the result is a feeble wave.

Now consider Fig. 210, where two waves of slightly different wave-length are shown, which are to be combined. Supposing we were considering the result in the position marked A, we see that there both waves are in the same phase, thus producing a strong combined wave. But these waves are travelling along with the velocity of light, therefore in an extremely short time the parts shown at B will have arrived from A, and as these parts are in opposition to each other we shall now have a very weak resulting wave. We thus see that if there is a difference in the wave-length of two combining waves, the resulting combined

wave in any fixed point, such as A, changes its amplitude in almost inconceivably short intervals of time between a maximum and a minimum; what takes place exactly corresponds to the "beats" which are heard when two musical notes of nearly

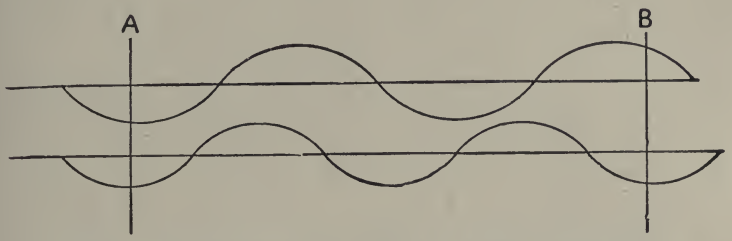


Fig. 210.—Combination of two Waves of different Wave-length.

the same pitch are struck simultaneously, with this difference, that the luminous beats follow each other with such rapidity that neither human vision nor instantaneous photography can differentiate; in both cases the average result is recorded, which a mathematical investigation shows to be the same as if the two waves acted independently and did not interfere with each other at all. Further, if we consider that about 500 billions of light vibrations pass a given point in one second, and that the human eye cannot separate light impressions following each other at intervals of one-tenth of a second, we can understand that it is extremely improbable that two independent luminous points should be sending out simultaneously light of sufficiently nearly the same wave-length to allow of visible interference effects. The two wave-lengths would have to agree within about 1 part in 50 billions, whilst the most powerful grating spectroscopes fail to distinguish between wave-lengths differing to the extent of one part in a million.

Accordingly the usual experience in all optical experiments is that only light which has originated in the same luminous point is capable of producing visible interference effects, and that therefore in studying interference or "diffraction" effects we must always consider light from one luminous point only. Such light is distinguished by the adjective "coherent." Therefore when different portions of light meeting at a given point are called coherent, this means that they are in a permanent relation as to phase and state of polarisation, and therefore capable

of regular interference effects, the inference being that such different portions of light have originally come from the same luminous point.

It may not yet be clear to the reader why it is important to distinguish coherent light from that originating in different luminous sources, and therefore vibrating independently. An example taken from microscopical practice should make this clear. When we try to get resolution of a difficult striation by using a small pencil of extremely oblique light, we see on looking down the tube two small patches of light at opposite ends of a diameter of the back lens of the objective. Every microscopist knows that one of these—usually much brighter than the other and of the colour of the source of light—is the direct light from the lamp ; the other fainter and generally coloured patch is the diffracted light. Now if these two patches of light were of different origin, they would act independently of one another. Each would send light into the eyepiece, uniformly lighting up the field, and if both acted together, the field would merely become brighter by the addition of the two intensities produced by the two luminous patches. In reality the two patches seen in the microscope are different portions of light from the same source which have been separated by the diffractive effect of the striæ in the object, consequently they are coherent, and when they meet in the field of the eyepiece they interfere and produce alternate maxima and minima of brightness after the manner shown in our Figs. 209A and 209B. The result is therefore that alternate dark and light stripes appear in the field of view which usually present considerable contrast by reason of another important property of light-waves: the brightness of the light corresponding to a given wave is proportional, *not* to its amplitude itself, but to the *square* of the amplitude, hence if we take the approximate proportions of our Figs. 209A and 209B, where the combining waves have amplitudes about as 2 : 3 and the combined waves amplitudes in proportion of the sum and the difference of these, or as 1 : 5, the difference in brightness between the darkest and brightest parts is as the square of the latter figures, or as 1 : 25! This accounts for the comparatively bold marking in the microscopical image which is usually seen on diatoms and gratings, even though,

on looking down the tube, the diffracted pencil appears extremely weak or perhaps barely visible.

The cases of interference which are most often met with and which are of the greatest importance are those which lead to so-called diffraction-phenomena; a brief explanation of these phenomena on the principles laid down above will therefore be of especial interest.

These cases may be stated thus:

Light from a luminous point passes through certain apertures—wanted, the intensity and phase of the light at any point beyond those apertures?

The solution is obtained by applying the Huyghenian principle and its extension by Fresnel.

According to the former, we obtain the light-effect at any point beyond a given wave-front by considering each point in the wave-front as a new source of light, but so that all of these points are at any moment in the same phase and state of vibration, and by combining the disturbances reaching the given point from all these points of the wave-surface, according to the universal rule stated above. Fresnel extended this principle to any surface containing the diffracting apertures, whether this surface coincide with the wave-fronts or not, by stipulating that the fictitious luminous points in that surface must have assigned to them the relative phases of the direct light reaching these points, and that the combined effect at any point beyond the surface must be deduced with due regard to these phase-relations.

I will not attempt to deal with the difficulties in connection with both these principles which have been raised on theoretical grounds, nor with the way in which they have been overcome; those who are interested in that are strongly recommended to look the subject up in Drude's *Theoretical Optics*.[1] Suffice it to state, that these investigations justify the applications of those principles which are here dealt with.

The application of these principles which is of most interest in the theory of microscopical vision is that which leads to the explanation of the peculiar effects produced by gratings and other regular structures.

[1] Drude, *Theory of Optics*, translated by C. R. Mann and R. A. Milliken. Longmans, Green & Co., 1902.

Limiting ourselves to the case where the light falling on the grating comes from a considerable distance and may be considered as practically consisting of plane waves, a grating will only allow light to pass through its apertures or slits arranged at regular intervals and in accordance with the principles laid down, the first effect will be that all points in the slit send out wavelets in all directions; these, however, being all caused by the coherent vibrations of one wave-front, start interfering with each other as soon as they meet, and the result depends on the phase-relation in which they meet.

Assuming first that the slits are exceedingly narrow, then there will be no sensible interference between the light starting from any one slit; we can therefore limit ourselves to considering what happens when the portions of light corresponding to the several slits have arrived at such a distance that the battle has been fought out and the final result of the interference has become established; and it is easy to see that in the direction of the original light-wave falling upon the grating, all the light starting from the apertures must again meet in the same phase and thus produce a strong light-wave—the direct light. If now we gradually consider points farther and farther away from the original direction of the light, it is evident that the portions of light from the various slits will here meet with a difference of phase, and will in general cancel each other more or less completely. But there must be directions at regular intervals where an exceptional state prevails. There must be two directions on either side of the direct beam where the light from any one slit meets that from its neighbour with a difference of phase equal to a *whole wave-length*; here then we shall again have reinforcement, and in the aggregate a strong light-effect. Similarly there will be other pairs of directions where the light from adjoining slits has a difference of two, or three or more *whole wave-lengths*; and in all these directions there will be further maxima of brightness. Usually the phenomena are observed with white light, and as this contains a great range of wave-lengths, the directions in which maxima are produced are different for different colours, the maximum effect of blue light, owing to its shorter wave-lengths, being obtained at a less distance from the direct light than the corresponding maximum for red with a much greater wave-length. The result

of it is that on either projecting upon a screen the light passing through a grating, or on looking through it at a distant white light, we obtain a direct image of the white light, flanked at regular intervals by *spectra* of increasing width but rapidly diminishing brightness, the familiar diffraction spectra.

For purposes of microscopical theory we must study, not only the direction and brightness of the spectra, but also their phase-relation, because we have seen that the latter is all-important in deciding whether the bringing together of the light of the different spectra at a given point will produce light or darkness. This will form a final example of the graphical method which we have dealt with in the earlier part of this chapter.

It is desired to determine the amplitude and phase of the

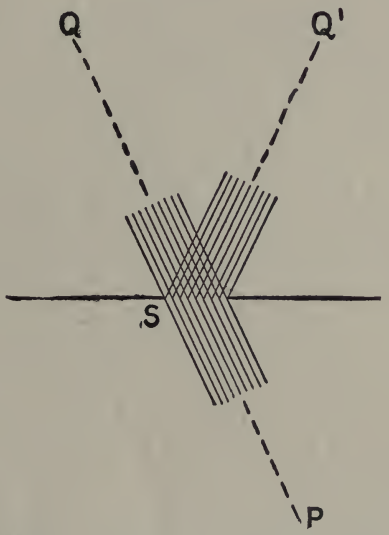


Fig. 211 —Formation of Diffracted Light.

light reaching Q′ from a slit S (Fig. 211) lighted from a distant point P, the amplitude to be compared with that which would obtain at point Q at the same distance from the slit as Q′ but in a direct line with P, and the phase to be referred to that which light from the centre of the slit would produce at Q′.

Both P and Q being at a distance which is assumed to be great as compared with the width of the slit, all the light will reach Q in the same phase, and we shall therefore get a resulting amplitude at Q, which is the simple sum of all the disturbances proceeding from the slit. But otherwise at Q′. For here we have obvious differences of the paths by which light from P through the different portions of the slit reaches Q′; hence there will be more or less weakening of the light at Q′ through interference. If we now divide our slit into a number of equal parts so narrow that the light from any one part may be assumed to reach Q′ in the same phase, we shall be able to combine the light from these parts *in pairs* of two parts equidistant from the centre of the slit by the simple process shown in Fig. 212. Such a pair close to the centre will have an inappreciable difference of phase, and we shall get a resulting amplitude nearly equal to the simple sum of the two, Fig. 212 (*a*). But if we take a pair with a considerable difference of phase, one being *behind*, the other an equal amount in *front*, of the light from the centre of the slit, then there will be interference. And an inspection of Fig. 212 (*b*) immediately shows a striking peculiarity; for as one wave-curve recedes just as much from any of the nodes of the central light as the other exceeds it, the displacements of the two waves at those nodes must *always* be equal to each other, but in opposite directions; on the principle of combination illustrated in Fig. 209 and again here, *the two waves will, therefore, invariably produce a node in the same position as light from the centre of the slit.* In Fig. 212 (*b*) there results a small combined wave still in the same sense as that from the centre. But proceed to Fig. 212 (*c*), where the difference of phase of either wave is more than a quarter wave-length as compared with the wave from the centre of the slit. We still get the same position of the nodes, *but these two waves produce a resultant wave of the opposite character to that of the wave from the centre of the slit.*

If we apply this process to all the successive pairs and then combine the resultants of these, we shall get the complete result; without going into the details, it may be pointed out that if the differences of phase between the extreme edges of the slit and its centre do not exceed a quarter wave-length,

all the resultants of the pairs are in the same phase and reinforce each other; for a wider slit, the pairs further removed from the centre combine to the opposite effect—hence the total light is weakened, and eventually becomes zero when the edges

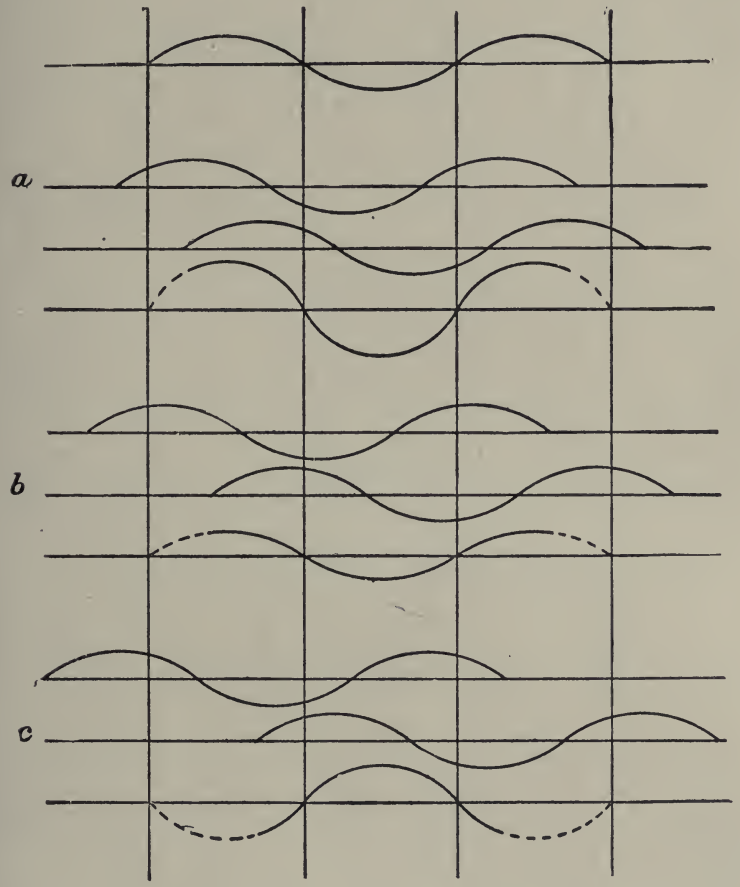


Fig. 212.—Illustrating Phase-reversal.

of the slit are half a wave-length out of phase compared with the centre. With still wider slits the light reappears, *but in the opposite phase.*

These alternations of phase in diffraction spectra were first pointed out, and their importance for the proper explanation

of microscopical images insisted upon, in a paper to be found in the *Journal of the Royal Microscopical Society* for 1904, pages 610–633, to which those who desire fuller information on the subject are referred. We will here merely state that the formation of correct images of gratings can only be accounted for by taking these phase-relations into careful consideration, and that it was indeed the impossibility of explaining the differentiation by the microscope between a grating of very narrow and a similar grating of very wide slits that led to their discovery.

CHAPTER XVI

THEORIES OF MICROSCOPICAL VISION

THE astronomical telescope was the first instrument to which the undulatory theory was applied with a view to determining the character of its image. Fraunhofer was well acquainted with the spurious disc and diffraction rings by which stars are represented in good telescopes, but it was left for Sir George Airy to solve the problem in a strictly mathematical way, and thus accurately to determine the size and brightness of the disc and successive rings with any given diameter and focus of a perfectly corrected object glass. Like every correct deduction from the undulatory theory, Airy's determination is confirmed by experiment, with this restriction: that Airy did not and indeed could not fix the size which the spurious disc would *seem* to subtend to a human eye. He did show that it had a fairly uniformly bright centre, and that the brightness then fell away quickly until it reached zero before rising to a second maximum in the first ring. Owing to physiological uncertainties the apparent size had to be determined experimentally and was found to be equivalent to a subtense of $4\frac{1}{2}$ seconds of arc with an object glass of one inch diameter, which means that with a telescope of this size two stars $4\frac{1}{2}$ seconds apart would appear to be in contact with each other and would thus mark the limit of true resolution. As regards objectives of larger or smaller aperture, both theory and experience agree in fixing this as governed by inverse proportion, *i.e.* a 2-in. objective will separate stars $2\frac{1}{4}$ seconds apart, a $\frac{1}{2}$-in. objective 9 seconds, or in a simple formula—

$$\text{Limit of resolution} = \frac{4\frac{1}{2} \text{ seconds}}{\text{Diameter of object glass in inches.}}$$

It must however be borne in mind that a telescope, if perfect, could nevertheless betray duplicity in still closer pairs, for the

corresponding spurious discs, though partly overlapping, would form an oval figure and would thus suggest two corresponding points of light.

It is not difficult to apply this limit to the microscope. For this purpose we must first get rid of the angular measure which is not used in microscopy, and replace it by linear dimensions.

Supposing we have an objective of D in. in diameter; its angular resolving power is then $\frac{4\frac{1}{2}}{D}$ seconds of arc, which means that the star-image produced by it, when viewed from the centre of the objective, would subtend that angle. Let the focal length of our objective be F in., then F is the distance just referred to from the centre of the objective to the star-image, and by plane trigonometry we find the linear diameter of the latter as $F \times \sin\left(\frac{4\frac{1}{2}}{D}\right)$ in. The sine of so small an angle may be put as $= \sin 1'' \times \frac{4\frac{1}{2}}{D}$, and as $\sin 1'' = \frac{1}{206265}$, we find the linear diameter of the spurious disc in inches for our objective of D inches diameter and F inches focal length $=$

$$\frac{4\frac{1}{2}}{206265} \cdot \frac{F}{D} = \frac{1}{46000} \cdot \frac{F}{D},$$

where the numerical factor has been rounded off in accordance with the limited accuracy of the accepted value of the resolving power.

Now it can be proved quite generally that where only a small field is required—and this is always the case in the microscope—the effect produced by a lens system does not depend on the exact composition of it, but that, on the contrary, we may substitute any imaginary lenses for it which may suit a particular investigation, provided that they do not disturb the course of the rays near the object and image respectively. Fig. 213 will make our meaning clear. Supposing that a certain

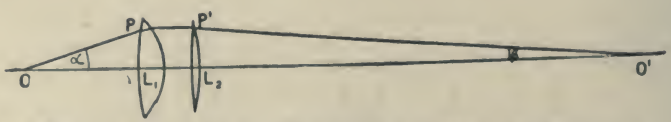


Fig. 213.—Airy's Limit of Resolution applied to the Microscope.

objective refracted the light from an object O in such a way that the incident ray OP reappeared—after traversing the objective—as the ray P^1O^1, then we may fill in the space between P and P^1 by any set of lenses which would produce this result. For our present purpose we imagine our objective composed of a lens L_1 just strong enough to make the diverging light sent out by O parallel, and a lens L_2 which converges this parallel light upon the conjugate point O^1. The lens L_2 therefore acts under precisely the same conditions as a telescope objective, and if its diameter be D inches and the distance L_2O^1 F inches, it will produce spurious discs (or circles of confusion) of $\frac{1}{46000} \cdot \frac{F}{D}$ inches in diameter. In the microscope the angles α and β which the marginal ray makes with the optical axis are of great importance, hence we will endeavour to introduce them into our formula. The angle β is always very small, so that there is no sensible difference between its measure in radians or its sine or tangent; hence we may say that we have $\sin \beta = \frac{\frac{1}{2}D}{F}$, whence, by transposing, $\frac{F}{D} = \frac{1}{2 \sin \beta}$, and this introduced into our formula for the size of the spurious disc, gives the latter as $= \frac{1}{92000 \sin \beta}$ in. The spurious disc and corresponding limit of resolution at O^1 when referred to the object O becomes diminished in inverse proportion to the magnifying power of the objective. In accordance with the sine-condition the latter is $= \frac{n \sin \alpha}{\sin \beta}$ if n is the refractive index of the medium surrounding the object.

Hence our spurious disc at O^1, $= \frac{1}{92000 \sin \beta}$ corresponds to a resolving limit in the object $= \frac{1}{92000 \sin \beta} \times \frac{\sin \beta}{n \sin \alpha}$. We see that $\sin \beta$, occurring in both numerator and denominator, crosses out; $n \sin \alpha$ is universally known as the measure of numerical aperture and symbolised by N.A., hence we find the limit of resolving power for a microscope objective according to Airy's theory—

$$\text{Limit of resolution} = \frac{1}{92000 \times \text{N.A.}} \text{ in.}$$

Thus an objective of N.A. 1 should resolve two points in the

object $\frac{1}{92000}$ of an inch apart; an objective of N.A. ·50 two points at $\frac{1}{46000}$ of an inch, etc.

The immediate significance of this result is that the microscope—if perfect—will show minute luminous particles as *spurious* discs of the approximate size given by the formula, and that it should just enable the observer to see *two* such particles separated if their distance agrees with the same limit. The potency of the limit finds expression even in the case of a *single* particle in the fact that, once the actual size of the latter is well below the limit, it is utterly impossible to tell from the appearance of the image what the real size of the particle may be, nor even its shape if it should really be an elongated body, but with its longest dimension below the limit of resolution. A brilliantly illuminated minute particle—say one with no dimension exceeding $\frac{1}{100}$ of the "limit"—would be represented by a spurious disc just as large and with the same distribution of light over its visible area as a much larger particle of less reflective power or more feebly illuminated. In either case one sees a little blot of light; what it is due to can only be found out indirectly, it cannot be decided by its appearance in the microscope.

Precisely the same remarks may be made with regard to *long* objects of "ultramicroscopical" *width*, such as the flagella of certain bacteria seen by dark-ground illumination; for when one determines (by integration) the light-distribution in the image of a luminous *line*, it is found to agree very closely with that determined for a minute luminous *area*, and therefore all that has been said above concerning the minute particle may be applied directly to the *width* of an elongated object.

Similar results are found by carrying out the integrations necessary to determine the character of the image corresponding to minute dark objects in a bright field. We get a grey smudge as the image of a minute black object, the width of the "smudge" agreeing closely with the limit of resolution, with no possible criterion by which a jet-black object of very minute diameter or width could be distinguished from a semi-opaque object of much greater size, so long as the real size remains

below the limit of resolution. Owing to the limited sensitiveness of the human eye (and of photographic plates also) to slight gradations of brightness there is, however, a real limit—though one not to be accurately determined—to the smallness of black objects which can be seen by the eye. It lies somewhere near $\frac{1}{20}$ or $\frac{1}{30}$ of the limit of resolution; that is to say, if an absolutely black object is narrower than this, then the deficiency of light in the part of the microscopic field where its image is formed is so slight (*i.e.* less than 5% about) as to escape the scrutiny of even a skilled observer.

We have devoted some space to this side of the question—more than it really is worth from the microscopist's point of view—because it has been used recently as the proverbial red herring to be drawn across the trail of true scientific progress. The subject is chiefly of interest to astronomers who *really* deal largely with self-luminous objects, and it is in astronomical literature that one finds most information on these matters.

It will be noted that the resolving limit thus deduced from Airy's theory agrees very closely with that determined by Professor Abbe on the basis of the diffraction produced by the object. It was arrived at from the same basis, but independently of Airy's work, by the famous Helmholtz in an excellent paper published in 1873, which also contained the first optical proof of the Sine-condition and many other interesting observations on the microscope and its action.

The paper here referred to had, however, a narrow escape from being left unpublished; for it so happened that when it had been set in type, but before it was published, there appeared an epoch-making paper by Professor Ernst Abbe, of Jena, on the same subject which shed an entirely new light upon the theory of the microscope. Helmholtz himself acknowledged the importance of this paper in an interesting postscript to his own, which latter he fortunately, although it would seem reluctantly, refrained from withdrawing entirely.

To realise the importance of Abbe's contribution to our subject we must bear in mind that up to then nothing had been done but to adopt the theory of the astronomical telescope to the microscope. Now the telescope is chiefly used on stars, which are self-luminous objects and which therefore really send out the strictly coherent light which Airy assumed in

determining the form of the image. The fact which had escaped the attention of physicists, including Helmholtz, was that the microscope is seldom, if ever, applied to self-luminous objects; that, on the contrary, objects are illuminated by a more or less distant source of light; that for this reason Airy's assumption is by no means fulfilled, and that therefore his result cannot legitimately be applied to the microscope.

This is the chief point underlying the Abbe theory; this theory of microscopical vision frankly accepts the illumination of objects by a distant source of light and indicates how the image seen is to be accounted for under these circumstances. Abbe's later papers show clearly that he considered this as the essential feature of his theory, and moreover that he realised that it applied not merely to very small objects, to which he had at first limited its application, but that it must supply the ultimate explanation of the images of all objects seen by borrowed light; even of fence-poles, as he tersely put it in a reply to one of his critics.

Unfortunately Abbe, like many original thinkers, greatly disliked writing long accounts of his work; his own publications on the subject of his theory are limited to a few papers, which moreover assume a very high degree of physical and optical insight in his readers, and which have in consequence been misunderstood and misinterpreted in all sorts of extraordinary ways by critics falling below the assumed standard of knowledge.

The result of all this has been that, while the Abbe theory has never been attacked by a competent physicist, there have appeared from time to time elaborate papers by others trying to prove that it was entirely erroneous; the "it," however, was never the Abbe theory as put forward by Abbe; "it" represented the particular author's way of misunderstanding Abbe's papers, or accounts of them in text-books.

The real difference between Abbe's theory and all others is that whilst the latter start from the object and *assume* this—consciously or unconsciously—to be self-luminous, the former begins *at the source of light.*

We saw in the preceding chapter that the light vibrations of any luminous point act independently of, and are incapable of interfering with, those from any other luminous point. Hence,

when there are a number of luminous points acting simultaneously, the resulting light effect in any given point is the simple sum of all the effects produced by the individual luminous points; to proceed logically, and in accordance with the fundamental principles of the undulatory theory, we must therefore first determine the effect produced by a mere point of light; we can then proceed to an extended source of light by repeating the operation for each point of it in so far as there may be any difference between the effects produced; we thus obtain the amount of light transmitted to any point in the final image from all points in the source of light; the sum (in reality, a multiple integral) thus formed is the true brightness of that particular point in the image.

Stated in this abstract form the Abbe theory looks rather formidable; it is, however, a direct and inevitable deduction from the laws of the undulatory theory, and as that theory—far from losing ground—is daily becoming more firmly established, the Abbe theory must share in this security from attack.

It is obvious that in the vast majority of cases the problem is too complicated for rigorous solution, in fact we may go further and say that the strict solution is generally impossible; for this simple reason, that the object itself modifies the light, and, as the final structure of natural objects is unknown to us, we cannot possibly determine how and to what extent these structural elements may obscure, retard, accelerate, or polarise light. All we can do is to deal with selected simple cases and to study the nature of the image obtained in such cases under various circumstances; from the results obtained we may then form some idea as to how much faith we may have in the everyday images of natural objects.

A few simple examples of this kind must therefore next occupy our attention.

Let L, Fig. 214, be a luminous point, OO′ an object lighted

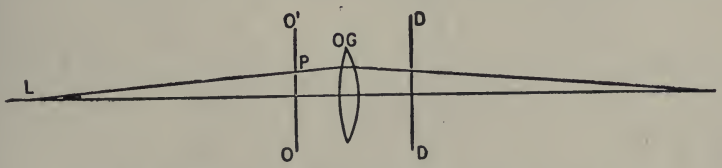


Fig. 214.—Isolation of the Direct Light passing through a point.

by it, and OG the object glass of a microscope focussed on the object OO′. If we fix our attention on a point P of the object, we see that but a single ray of light from L can pass through it; if the formation of the image were due to this ray passing onwards through the microscope, according to the rules of geometrical optics we could place a diaphragm D with a very small aperture which would nevertheless pass the single ray with plenty of room to spare, and which should therefore have no effect on the image. Such a diaphragm would however cut down the useful aperture of the objective to a very small diameter, and both Airy's theoretical determination and practical experiment would therefore show us that the image of the point P under these conditions would become a very large diffusion-disc. But in making the experiment we should also find that the moment we withdraw the diaphragm D, and thereby expose the full aperture of the objective, the image of our point is immediately sharpened up. There is therefore something wrong with geometrical optics in this case, and that "something," as Abbe was first to point out, is the diffraction of the incident light by the object.

We have indeed already learnt in the preceding chapter that whenever we cut down a beam of light by the interposition of a diaphragm, there results a spreading out of the light beyond the geometrically determined limits, owing to interference phenomena; if, for instance, we assume our point P to be a minute aperture in an otherwise opaque plate, theory and experiment show that the incident light, so far from being reduced to a single ray, is spread out so that light proceeds in every direction from the tiny aperture, and as all this light is due to the vibrations sent out by the one luminous point L, it is all coherent and forms regular spherical waves proceeding from P as their centre; owing to its diffractive effect our minute aperture has indeed become, to all intents and purposes, self-luminous, and the microscope will form an image of it in accordance with Airy's theory, viz. a small spurious disc surrounded by faint diffraction rings. But this case of a *solitary* and *minute* aperture is the only one where the Abbe theory leads to the same result as that of Airy and Helmholtz. As soon as our aperture exceeds a very minute diameter (*i.e.* less than half a wave-length) the diffraction effect becomes more

complicated, consisting of a central cone of light surrounded by a number of rings, in which latter moreover there is that alternation of phase which we proved to exist in all these cases in our last chapter. Hence the Abbe theory leads us to realise a state of affairs very different from that assumed by Airy and Helmholtz. Again, if instead of one aperture we have a number of them fairly close together, then there is further complication, for the light having first suffered diffraction by each individual aperture, the resulting vibrations issuing from the several apertures immediately afterwards become commingled and again interfere, with the result that the light issuing from such an object is broken up into a number of more or less isolated beams, which, when they are clearly separated, form what are known as diffraction spectra.

The explanation of microscopical images, according to the Abbe theory, thus resolves itself into two parts: first, to determine the result of the diffraction of the incident light by the object; secondly, to ascertain the effect produced in the plane of the image when the diffracted beams meet there; and, as we stated above, this is possible only in selected cases of simple regular structures.

Professor Abbe naturally was aware of this; he therefore had a few suitable objects prepared for him by the firm of Carl Zeiss, with which he was prominently connected. These objects included simple and crossed gratings, ruled in silvered glass, which give strong diffraction spectra easily accounted for by theory. A set of three has indeed been sold by the firm for a long time under the name of the "Diffractions-platte." With the aid of these objects Professor Abbe showed conclusively that the image obtained depended entirely on the combination of spectra admitted into the microscope, and that it indeed always agreed with theory, even in cases where the similarity of object and image was utterly destroyed by the exclusion of suitable spectra. Thus he demonstrated, for instance, that the number of lines in a simple grating might be shown doubled, trebled, or quadrupled by excluding one, two, or three of the spectra next to the direct beam; that the crossed lines of the other gratings might be shown one set at the time, either at their right distance apart, or again doubled or trebled; nay, that with these crossed gratings one might produce a system

of lines having a totally different direction from those known to exist in the object, and a different distance apart as well.

Probably the description of these experiments in text-books without adequate comment has done more to make microscopists rebel against the Abbe theory than any other circumstance. These experiments were indeed admirably devised to convince the highly trained students of the University professor that diffraction by the object was the true source and fountain-head of microscopical images; but put before less scientific observers without proper comment, the same experiments had an almost opposite effect. These observers knew, or at least felt, that such utter dissimilarity between object and image did not occur in everyday practice; that, on the contrary, the image seen with a low-power object glass was only added to and *improved* in its *finer* details with a stronger objective, but was never replaced by a totally dissimilar image; they thus became distrustful, and although they were unable to "make a hole in the theory," or to find any physicist who would undertake the task, they followed the lead of a well-known text-book by sitting on the fence, "waiting for further light."

We may as well state at once that it has recently been pointed out by Mr. Conrady that all the "most startling nightmares"—to use his picturesque description of them—produced with the Abbe "Diffractions-platte" are obtained under conditions which cannot possibly occur in the normal use of the microscope; they all require the use of suitably cut diaphragms behind the objective by which certain diffraction spectra are *deliberately suppressed* that would otherwise enter, and which, if admitted, would at once cause the "nightmares" to vanish and to be replaced by an image reasonably similar to the object. If the descriptions of the experiments in earlier text-books had pointed this out, a vast amount of misunderstanding and scepticism would have been nipped in the bud.

Before examining the first of the objections raised against the Abbe theory it is only fair to point out that Abbe's first paper of 1873 [1]—the one which impressed Helmholtz so deeply—was largely concerned with matters of optical theory, and men-

[1] "Beiträge zur Theorie des Mikroskops und der mikroskopischen Bilderzeugung," Max Schultze's *Archiv für mikroskopische Anatomie*, ix. pp. 413-68 (1873).

tioned only quite briefly the experimental investigations by which the author had convinced himself of the great importance of the diffraction produced by microscopical objects and the conclusions he had arrived at; it would indeed seem that this first paper did not produce a very deep impression in those readers who could not bring to bear on it the penetrating insight of Hermann von Helmholtz.

The first elaborate attack on Abbe's theory appeared in 1880 in the form of a paper in a German medical journal by a Dr. Altmann.[1] This gentleman—having only the above-mentioned brief account of the Abbe theory to base his opinion upon—claimed, perhaps with considerable justification, that Abbe had based very far-reaching deductions on very little evidence. He then attempted to reconcile the theory advanced by Helmholtz in his paper of 1873 with the conditions prevailing in the ordinary use of the microscope. The task thus attempted was to account for what has been aptly called *the mystery of the dark space*—viz. that when we work with a narrow cone of light, and when frequently, on looking down the tube of the microscope, there is only seen a small patch of bright light, surrounded by a wide dark space apparently devoid of light, that then experiment nevertheless shows that this dark space plays an important rôle; for, in accordance with what was stated above, if we reduce the clear aperture of the objective so as only just to admit that bright patch of light, definition is immediately ruined and much fine detail completely lost. The conclusion is irresistible that the apparently dark space contributes light which, although sometimes very faint and indeed invisible, is of the utmost importance in building up the image. Abbe had claimed that this light in the "dark" space was due to diffraction in the object. Altmann tried to make out that it was lens-action by small structural elements which principally accounted for that faint light. But, apart from this, he claimed that, whatever the light in the "dark" space might be due to, there was no need to depart from the Helmholtz theory—*i.e.* from the adaptation of the theory of the telescope to the microscope, for, said Dr. Altmann, whatever the origin of this light may be, we have the objective more or less com-

[1] "Zur Theorie der Bilderzeugung," *Archiv für Anatomie und Physiologie* (*Anatomische Abteilung*), 1880, pp. 111–84.

pletely filled with light, though of varying intensity; all we therefore have to do is to carry out the integration—which Airy and Helmholtz performed for a uniformly filled objective—so as to take the intensity and distribution of the light into account. We shall thus arrive at a modified spurious disc or diffusion-disc, as he called it, and can consider the image as built up of overlapping diffusion-discs of this modified type.

Such, in brief, were Dr. Altmann's criticisms of the Abbe theory and his suggested rival theory. Particular interest is attached to both because they brought an immediate reply[1] from Professor Abbe, in which the newly born theory of Dr. Altmann fared very badly indeed.

In defence of his claim for diffraction rather than refraction as the cause of the scattered light, Professor Abbe described a number of carefully conducted experiments with glass threads in oil and grooves of determined shape cut with a diamond in glass slips and filled with oil for which the possible intensity and degree of deflection of the refracted light could be accurately computed. These experiments showed that the objects scattered light far beyond the limits to be accounted for by their lens or prism action. Professor Abbe indeed stated that he himself only gave up the refraction theory when he had convinced himself by these and similar experiments that it could not explain the mystery of the dark space, and that only then he sought and found the explanation in diffraction by the object. He then further elaborated his former brief account of his theory, and for the first time dealt with the importance of distinguishing between self-luminous objects and those illuminated by light from a distant source. We have anticipated much of this in the earlier part of our brief history, and reference to that part will suffice to show that it is indeed quite impossible to assume with Dr. Altmann that the light at the back of an objective could be treated in the same way as if it had started in regular spherical waves from points in the object and was only distinguished from that assumed by Airy and Helmholtz by its intensity varying in different parts of the aperture of the objective. As we have shown, we are bound to begin by considering light from one point only so as to be sure of its being coherent and capable

[1] "Ueber die Grenzen der geometrischen Optik," *Sitzungsberichte der Jenaischen Gesellschaft für Medizin und Naturwissenschaft*, 1880, pp. 71-109.

of regular co-operation ; and we saw that when we do so we get only one "ray" of direct light passing through any one point in the object, which latter can only become distinguishable by scattering the light, generally by the process of diffraction. If there is a more or less extended brightly illuminated area visible at the back of the objective, it must on no account be treated as coherent and capable of co-operating after the manner of light from a self-luminous object ; it must be subdivided into the portions which are each due to one point of the source of light, otherwise we come into collision with the proper interpretation of the undulatory theory of light.

Curiously enough, speculation of much the same sort as that of Dr. Altmann formed the subject of an extraordinary paper which was read at a meeting of the Royal Microscopical Society in 1901. Apart from a highly curious account of the author's conception of the Abbe theory it contained nothing that had not been anticipated by Dr. Altmann twenty-one years earlier, and consequently we need not devote any further space to it.

We hope to have made it clear in our remarks up to this point that in our opinion the chief obstacle in the way towards the inevitable ultimate adoption of the Abbe theory has been the way in which the latter has been put forward. The rather sensational experiments with the "Diffractions-platte" have been put in the foreground, thus leaving the average student to think that here was the kernel of the matter, whilst the far more important position of the real Abbe theory as a direct and necessary deduction from the fundamental principles of the undulatory theory of light has been either omitted entirely or dismissed in a short introductory paragraph or chapter in the manner of Dippel in his *Handbuch*, of which, unfortunately, there is no English translation, although it contains the best and indeed "semi-official" account of the Abbe theory ; semi-official inasmuch as it had Professor Abbe's sanction.

In addition there always has been that yawning and unbridged gulf between the Abbe theory as described in the text-books on the one hand, and everyday microscopical practice on the other. In the "experiments" we use a small and remote source of light and all sorts of oddly shaped diaphragms behind the objective, whilst in serious microscopy we shun both these

things by using as large an illuminating cone as the object will bear and by carefully refraining from interfering with the natural round aperture of our objectives.

We are glad to think that this gulf is at last being bridged; that at last attempts are being made to bring the Abbe theory into touch with everyday microscopical manipulation and observation; and that these attempts are free from any vainglorious effort to rob Professor Abbe of the credit for his greatest achievement. For although it is undoubtedly true that Professor Abbe has given only the briefest accounts of his theory, and has indeed only outlined its scope, we feel that any theory which recognises the difference between self-luminous objects, such as sun, stars, and nebulæ, and objects lighted from a more or less distant source, must have his name connected with it, as he undoubtedly was the first to recognise and to insist upon this fundamental difference and its importance in connection with the correct interpretation of optical images. And just as we speak to this day of the Copernican system of the world, although Copernicus wrongly described the planets as moving in circles, and although he could not give the slightest reason for their moving in this manner, so we feel that Abbe's name must be attached to any theory of vision which recognises the fundamental difference between self-luminous and artificially lighted objects.

The most distinguished worker in this field was Dr. Johnstone Stoney, F.R.S., who, besides much experimental work on resolution and its limits, first drew attention to a very important peculiarity of artificially lighted microscopical objects which extends the sway of the Abbe theory into regions in which it would not otherwise apply. We refer to so-called critical illumination, in other words, to those cases where by the use of a highly corrected and carefully focussed condenser a sharp image of the source of light is thrown upon the object under observation. Under these conditions the light from each point in the source of light is concentrated upon the corresponding point of the flame-image and therefore also on one point of the object; and as each point of a source of light sends out independent vibrations, one should expect that the points of an object so illuminated would also send out independent vibrations and would thus behave as if the object itself were self-luminous.

Dr. Stoney made the startling discovery that this is not the case. We may be ever so careful in the choice and adjustment of the condenser so as to realise the conditions of critical illumination to the fullest extent; if the object is of sufficiently regular structure to give rise to recognisable diffraction spectra, we always shall be able to observe them in exactly the same positions and relative intensity as if a cone of direct skylight of the same angular extent were allowed to fall upon the object, when Abbe's assumption would of course be fulfilled.

Dr. Stoney has explained this anomaly by claiming physical reality for a well-known mathematical theorem.

It can be shown mathematically that no matter how complicated a train of simultaneous light vibrations may be, it is always possible to determine a set of plane waves which would be in every respect a perfect substitute for the vibrations under consideration. There is however nothing either in the mathematical proof of this theorem or in the fundamental principles of the undulatory theory that would lead one to think that this substitution would ever become a physical reality; on the contrary, it has always been looked upon as a most improbable suggestion. The discovery of Dr. Stoney would however seem to call for the acceptance of this theorem as a physical reality. The continuous existence of the diffraction spectra under critical light seems impossible to explain without such an assumption; and in that event the Abbe theory would apply to such cases in precisely the same manner as it does under less carefully regulated illumination.

Another indefatigable worker in the field of the theory of microscopic vision is Mr. Julius Rheinberg, whose work is chiefly of the experimental order and has resulted in many discoveries of great interest in themselves and of considerable importance from the theoretical point of view.

In recent years Mr. Conrady read several papers before the Royal Microscopical Society with a view to bringing forward the real significance of the Abbe theory in accordance with our brief account of it in this chapter. In these papers Mr. Conrady has also begun the task of demonstrating how the image of a microscopical object is built up according to Abbe's theory and of showing that, when applied to the ordinary working conditions of the microscope—in contradistinction to the artificial

conditions prevailing in the usual experiments with the diffraction-plate—this theory fully accounts for all peculiarities of the image. It is a particularly satisfactory result of these investigations that they lead to the conclusion that the possibility of gross deception by a properly used microscope is extremely remote, and that this instrument may be depended upon to almost the same extent as the telescope to give correct indications of everything *within its limit of resolving power*. But this only applies on condition (1) That the natural round aperture of the objectives is not in any way obstructed or interfered with by stops or irregular diaphragms placed anywhere between the object and the eye; (2) That a reasonable amount of well-centred and uniformly bright illumination is used, that is to say, that on looking down the tube a perfectly round disc of light is seen, equally bright over its whole area, and concentric with the back lens of the objective, which latter may be filled with light to any extent from a minimum of perhaps one-third of its diameter up to nearly its full diameter, according to the nature of the object; and (3) That dark-ground illumination is *avoided* for *critical work* of any description, as it can easily be proved that this kind of illumination is capable of giving false images of a decidedly dangerous character.

This chapter may aptly be concluded with a few general remarks on the nature and requirements of the theoretical investigations that it deals with. As there is now only one theory of light, namely the undulatory theory, the theory of any particular optical instrument, such as a microscope, simply means the application of the undulatory theory to the particular conditions prevailing in that instrument, with a view to explaining the effects it produces and, if possible, giving indications as to the direction in which the instrument might be improved. The *test* of such a theory must always consist in proving agreement between the results predicted by it under perfectly defined conditions and direct observation under the precise conditions assumed in the theoretical investigation. For the telescope and for the spectroscope, Sir George Airy and Lord Rayleigh respectively have supplied theories which fully satisfy these requirements. In the case of the microscope, Abbe was the first man who realised that here peculiar difficulties come in which are not present in the other two. One of these difficulties

arises from the nature of the illumination, and this we have already dealt with in earlier parts of this chapter. But another difficulty consists in finding *objects* to which the undulatory theory can be successfully applied, and this difficulty also was first realised and overcome by Abbe. It arises from the fact that in the microscope we endeavour to detect, by means of light-waves, minute structural details which are themselves as small as or actually smaller than the wave-length of light. As it is possible, nay probable, that *all natural objects* have ultimate structural elements of extreme but unknown minuteness, and possess corresponding irregularities of surface, of outline, and of distribution of substance, it is a matter of simple logic that if we were to use any natural objects to test a microscopical theory, we should be at once moving in a vicious circle, for we should have to start with assumptions incapable of being proved as to the ultimate structure of the object, and any conclusions derived from such unproved assumptions would of necessity be quite valueless in either establishing or rejecting a theory. It was on account of this obvious objection to natural objects that Prof. Abbe limited himself to artificial ones, the nature of which could with considerable confidence be claimed to be completely known, so that the images they should yield could be correctly calculated by the formulae of the undulatory theory, and a test of the theoretical result obtained which would have real value. The objects which Prof. Abbe judged to be sufficiently determinate as to structure were glass-threads, either in air or immersed in liquids, and especially coarse systems of lines ruled in a thin deposit of silver or of smoke on polished glass, care being taken to remove the silver or carbon only, without scratching the glass. *Fine* gratings ruled with a diamond on either glass or metal can *not* be treated as known structures for two reasons, the first being that the shape of the diamond point is unascertainable, and the form of the grooves produced by it correspondingly in doubt; the other that there is every reason to assume that, in making these fine rulings, the whole of the original surface of the plate is apt to be ploughed away, so that differences of level are produced, which must affect the action of the grating. This uncertainty of fine gratings has been abundantly proved by the discordant results frequently obtained with them in attempting to determine absolute wave-lengths.

This point, as to the difficulty of finding suitable objects for theoretical purposes, is worth emphasising, because there have been several would-be theoretical papers in recent times which dealt with observations of finely marked diatoms, or of fine rulings on glass. For the reason stated, no value whatever attaches to any *theoretical* deductions supposed to be drawn from such observations, however interesting the latter may be from the practical microscopist's point of view.

CHAPTER XVII

ACCESSORY APPARATUS AND HOW TO USE THEM

A Metal Holder for the Metallurgist

THE difficulty of holding a piece of metal for studying its fracture or structure often arises, *especially when the observer is not provided with a metallurgical microscope.* This has been met by an ingenious arrangement (Fig. 215) by Messrs. Watson &

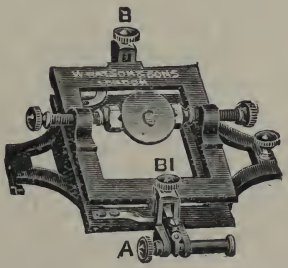


Fig. 215.—A Metal Holder for the Metallurgist.

Sons, which we believe can be adapted to almost any microscope with little difficulty. It combines a metal *holder* and the means of *levelling* a specimen. Two screws with *rotating jaws* grip the specimen C, and if its plane be not at right angles to the objective it can be tilted to the desired position by means of the adjusting screws A, B, and B1. It should be mentioned this holder is also supplied in a simpler form, without any adjustments for levelling, if required. *Blocks* of metal can also be held in the jaws of this arrangement at *any* angle required and can be *rotated*, thus obviating the necessity for the long and tedious process of cutting and mounting specimens.

An Illuminator for Metallurgical Specimens by Mr. Stead

A very simple and yet very effectual method of illuminating metallurgical specimens when employing low-power objectives, such as a 1, 2, or 3 inch, is shown in Fig. 216. The principle of illumination will be obvious by inspecting the woodcut. The metal box has one of its sides cut at an angle of 45°, this being faced with a small square of glass the surface of which is illuminated by means of a lamp or small bull's-eye condenser; the circular collar pushing on to the body of the objective.

Fig. 216.
Stead's Illuminator.

The Diffraction Micrometer Eyepiece

This micrometer is not only different in general construction from the regular screw-micrometer, but the scale itself and the method of reading sub-divisions are novel.

In the ordinary micrometer, whole divisions are read on the scale and sub-divisions on a drum attached to the milled head which carries the moving measuring wire. In this new diffraction pattern, the sub-divisions are read directly on the scale by a simple and absolutely precise means.

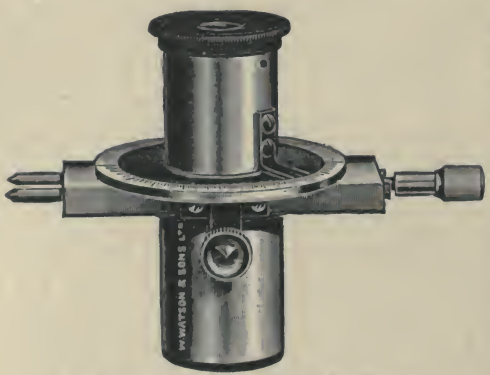


Fig. 217.—The Diffraction Micrometer.
By Mr. J. W. Gordon.

To save time and the unnecessary use of a fine screw, the frame carrying the travelling hair can be moved across the greater portion of the field by sliding. When it is approximately in the position required, the micrometer screw is brought into use. This materially assists in rapid working.

The scale is of special design. The extremities of the object under measurement are taken by means of the moving web in the usual way, and for

ascertaining fractions of a whole division a diffraction grating is supplied in the eyepiece cap. By rotating this cap, a superposition of the image of the micrometer wire is produced upon the image of the adjacent scale division. Attached to the cap is a reader, which moves against a portion of a divided circle when the eyepiece cap is rotating. These divisions represent one-hundredths of a whole division. By reference, therefore, to this divided circle the fractions can be read immediately.

The eyepiece cap is removable, so that ordinary observations may be made independently of the diffraction plate when desired. When the diffraction plate is in use, the resulting images flash out dead black at the moment of superposition, and thus make the taking of an exact reading in this way not only easy but certain.

Gauges for Cover-glasses and Slips

To know the thickness of cover-glasses and slips is always a convenience, but with objects requiring high-power dark-ground illumination it is a *necessity*, that is if the best results are aimed at.

Ciceri Smith makes a direct vision micrometer of great excellence (Fig. 218). It is seen, by examining the illustration, that in taking a measure all that is required is to place the object between the "anvil" (or hexagonal nut) and the spindle, and to move the latter, by means of the thimble attached to its end, when the windows will show the thickness at a glance. To prevent pinching too strongly, the thimble is only "friction-tight," and readily slips in its fitting if too great a force be employed; hence fracture of a cover-glass very rarely occurs.

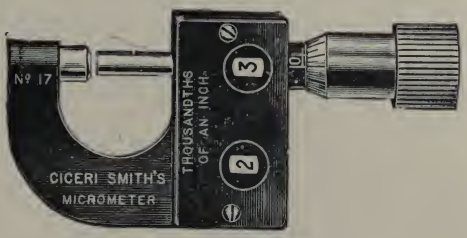


Fig. 218.—Ciceri Smith's Micrometer.

Zeiss's arrangement shown in Fig. 219 is of different design although direct reading. We can testify from long use this little machine is excellent in every respect. Turning the handle opens the jaws, which close by themselves on the slip or cover, the needle showing the thickness in terms of millimetres and

inches. Care should be taken, when measuring a slip, that it does not drag on the jaws as it rests on the table (for the jaws will not hold it suspended as they do a cover-glass), for, if this be the case, a false reading will inevitably result. If the needle when it flies back does not come to zero exactly, it can be set by the adjusting screw underneath the wooden mount. The measures shown by the dial can be verified by using platinum wire which is obtainable of standard thicknesses.

Fig. 219.—Zeiss's Cover-glass and Slip Micrometer.

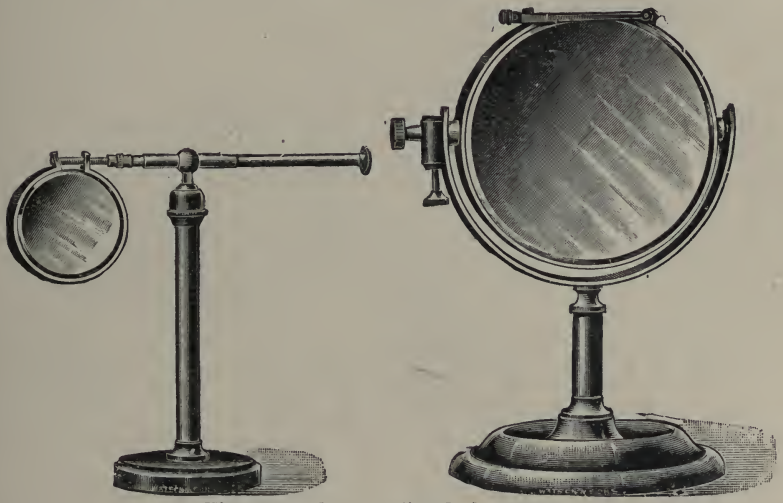
Goniometer

A Goniometer is an instrument for measuring angles, but more particularly those formed by the faces of crystals. It is also employed for the purpose of determining the separation of the optic axes in bi-axial crystals. Messrs. Swift & Sons make a Stage Goniometer which is very efficient. It is fixed to the revolving stage, and is used as follows: A section of the mineral cut perpendicularly to the principal axis is placed on the forceps of the instrument (when fixed to stage) and so arranged that the line joining the optic axis is inclined 45° to the crossed Nicol's prisms (these being set parallel to the cross-wires in an eyepiece especially so provided) while the same line is at right angles to the direction in which the forceps point. The pointer is then turned round until the darker part of one of the brushes covers the intersection of the cross-wires, when a reading is taken of the amount of rotation. The pointer is then turned in the opposite direction until the darkest part of the other brush covers the intersection of the cross-wires, when a second reading is made. The difference between the two indicates the apparent angular separation in air. Much the same method is adopted when measurements are taken in oil; but the microscope is placed *horizontally*. Very small sections of minerals are attached

by wax to the point of the forceps or to a needle that is supplied to take their place.

Kingsford's Troughs

Mention has been made in the text, when discussing the use of colour-screens, of a cheap and useful trough to hold solutions as colour-filters for contrast purposes or for obtaining approximately monochromatic green illumination. The arrangement suggested by Mr. Kingsford meets every requirement of the microscopist. It consists of an outer circle of brass, drawn together at the top by means of a screw or screws. The brass circle is lined with india-rubber, and blocks of the same material



Figs. 220 and 221.—Kingsford's Troughs.

are so placed as to keep the glass faces, which are circular, in position. These glass discs are gripped by the india-rubber when the tension on the outer metal band is increased by tightening the screws, and an absolutely watertight fitting is the result. A distinct advantage of the invention is that every part can be easily taken to pieces to clean by merely releasing the screws, and either of the glass plates, if accidentally broken, can be as readily replaced for a few pence. They are made and sold by Messrs. Watson & Sons at a very moderate cost, and are manufactured in two forms (Figs. 220 and 221).

Photography with the Microscope

To obtain results similar to the well-known photomicrographs by highly experienced artists such as Messrs. Bousfield, Freshwater, Green, Lees-Curties, Nelson, Norman, Pringle, Rodman, Taverner, and many others, requires a first-class equipment; but a more modest arrangement may be of some service to those who merely desire a memento of some special position in a slide and who are satisfied with a photograph of not so finished a character. For this purpose, where great magnification is not

Fig. 222.

Fig. 223.

Cameras for fixing on the Microscope.

required, several manufacturing opticians make a little portable apparatus which is arranged to be dropped on to the top of the microscope, being held there by some suitable means. Messrs. Swift & Son sell a very neat and useful arrangement of this kind (Fig. 222). It is formed in aluminium, being provided with a clamp at one end whereby it is attached to the draw-tube, whilst a camera is fixed on to the other. In a laboratory, when a quickly taken photograph is wanted, this device—or another made in mahogany by Messrs. Watson & Sons (Fig. 223)—is very convenient. Messrs. R. & J. Beck also sell an arrangement devised by Mr. J. W. Gordon (attachable

to any microscope) with which useful photographs are procurable with ease.

It is not within the scope of this book to explain the process of taking photomicrographs, so it must suffice to mention in what follows the general principles involved in so doing.

The rays of light coming through the ocular, when visually observed by the microscopist, are converted into a virtual image of the object which is apparently seen projected upon a plane at a distance of ten inches from the eye, being magnified according to the optical arrangements obtaining at the moment. When, however, a photograph is being taken, these rays are allowed to recross and fall upon the ground glass of the camera upon which they are focussed by the coarse or fine adjustments of the microscope in the ordinary way. If the ground glass be ten inches away from the eyepiece the image is of the same magnification (approximately) as the observer would see were his eye at the instrument, and the amplification is proportionately increased as this distance becomes greater and greater. If, however, the screen be set nearer to the ocular than ten inches, the magnification is proportionately decreased in a similar fashion. This being understood, it is evident that if these cameras are made very short and are fixed on to the very end of the draw-tube and the photograph taken in this situation, the resulting picture is sensibly less magnified than the object would appear to the observer when looking down the microscope. To make the amplifications equal, or anything like so, a much higher ocular must be employed, which in some cases may not be desirable. To avoid the very small image of which we have just spoken, in most of the modern arrangements the length of the camera reaches very nearly to ten inches. But it is here that a great difficulty arises, for as the arrangement is made longer and longer, so is it more and more inclined to vibrate when being used, which upsets the definition, and so of course spoils the final result. Some manufacturers have come to the opinion that with these little cameras it is best not to have their length more than about six inches, and, taking all in all, perhaps their judgment is correct. The only other way of getting over the difficulty is to use an objective of higher power, as the short camera reduces the magnification (as seen in the ocular) to about that required for the photograph.

The Davis Diaphragm

Although cutting down the substage iris diaphragm lowers the numerical aperture of the objective to any amount required; still there are times, especially whilst employing low powers and dark-ground illumination, when it may be inconvenient to employ it for that purpose. A very useful arrangement, consisting of an enclosed iris diaphragm, may then be substituted which is affixed to the tube of the microscope by one end, whilst the other serves to hold the objective. It is called, after its inventor, the Davis Diaphragm. Many such are upon the market; but the fault of several is that the case containing the actual diaphragm is only sprung together, and, in consequence, is very apt to come apart when taking the apparatus off the instrument. The leaves of the iris then fall out, and it is no easy matter to return them to their original positions. Besides this, not a few are made very imperfectly in one particular—viz., the iris does not shut concentrically with the optical axis. The consequence is that the back lens of the objective is not closed "truly"; in other words, when the diaphragm is, say, half shut, it will be found on looking down the tube that part of the outer zone of the objective is in use on one side whilst the middle zone is all but closed on the other. Such a state of things is very apt to spoil the action of the objective.

Fig. 224.—Davis Diaphragm.

To ascertain if this apparatus be well made, place an inch on the nosepiece of the microscope and a single diatom, like an Aulacodiscus on the stage, arranging it so that it shall lie in the centre of the field as seen when looking down the eyepiece. Now remove the inch fixing the Davis in its place, and attach the lens to its lower end. On looking down the microscope and refocussing, the diatom should be still central. Having nearly closed the leaves, rotate the Davis diaphragm in its own collar, about the axis of the instrument; the diatom should remain central.

It should be pointed out in addition, that the lower portion of

the arrangement, being made to revolve in its own fitting about the axis of the microscope, is of great service (not being found in the original model). Besides of use as explained in the previous paragraph, it serves the useful purpose of arranging the apparatus so that the handle operating the leaves can be placed at the front, at the sides, or at any position most convenient.

Those who employ this arrangement should bear in mind that the tube-length of the microscope is increased by its use, hence that the draw-tube needs adjustment by pushing it in an amount equal to the length of the apparatus.

The Diamond Cover-glass Marker

It has been shown elsewhere in this book how to register any particular place of interest in a given slide by aid of the verniers so that it can be readily found on any future occasion. It is obvious, however, this method is of no avail if the specimen has to be used upon a different instrument, as occurs when sending it away to another microscopist to examine, or to a laboratory to be photographed. Hence, to indicate the exact spot of interest, or the precise field of view, so that there cannot be any mistake, is always found to be a very troublesome and uncertain matter. To meet this difficulty, the "Diamond Cover-glass Marker" has been devised. As shown in Fig. 225, it consists of a "dummy" objective provided with a pin projecting from its lower extremity which is *not* centrally placed with respect to the optical axis. This pin is very fine towards its end, and the actual tip is furnished with a minute diamond. The objective in use at the time being removed, the "dummy" is substituted, and lowered very boldly upon the cover-glass, no injury resulting thereto because the pin rests, within the mount of the marker, upon an *exceedingly* delicate spring. The milled collar around the body of the "dummy" being now rotated, causes the eccentrically placed diamond point to revolve, and thereby describe and engrave on the cover-glass a minute ring which encloses the special portion of the specimen recently viewed by the objective. This delicately marked circle on the

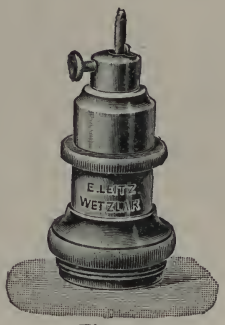


Fig. 225.
Cover-glass Marker.

cover-glass can be readily recognised by a second observer who in the first instance uses a low-power objective to locate its exact position.

It can be easily understood when the arrangement is employed to follow the use of a lower power, the diameter of the ring is required to be of sensible size so as to embrace the large field of view exhibited by such an objective. The exact limit required is obtained very readily by turning the little adjusting-screw seen projecting from the side of the mount; this, by bringing the diamond point farther away from the optical axis, produces—when the milled ring is turned—any increase of diameter of the enclosing ring that may be desired. On the other hand, when the marker is employed in conjunction with a high power, such as a $\frac{1}{12}$th, for example, which has a very small field of view, it becomes requisite to restrict the size of the enclosing circle as much as possible. This is brought about by turning the little screw in the opposite direction as far as it is made to move, which by bringing the diamond as near as can be to the optical axis forces it to describe an exceedingly minute circle when the milled ring is turned.

Some little difficulty, however, may be experienced from time to time by microscopists in knowing *how much* to turn this little adjusting-screw so that it shall (without immediate experiment) make a ring on the cover-glass exactly suitable to the objective in use. To overcome this Herr Reichert adds a small micrometer that records certain figures which by direct experiment will furnish the user with the requisite information. It is best to try this micrometer with different combinations and to record the figures which produce circles of the correct diameter for each objective. When using any combination at a future time then, the microscopist knows at once, by setting the micrometer to the previously ascertained figures, that a circle of suitable dimensions will be engraved upon the specimen on the stage. This little arrangement is shown in Fig. 226.

Fig. 226. Reichert's Cover-glass Marker.

The following hints may assist the microscopist when learning to use either of these little arrangements :—

1. The diamond point is to be pressed somewhat *firmly* on to the cover-glass by lowering the tube of the microscope a sensible amount; if this be not done sufficiently it may fail to mark.

2. It is possible a cleaner cut can be made when revolving the milled ring one way than when it is turned in the other; the better of the two when discovered should be adhered to afterwards as it preserves the life of the diamond longer always to move it in the same direction.

3. One complete revolution should be sufficient; two are apt to cause the formation of several microscopical splinters which settle on the glass around and upon the actual circle described.

4. When used after an immersion objective the oil should be first gently soaked off the cover-glass by applying one or two little pieces of blotting paper in succession, great care of course being taken not to move the slip whilst so doing.

5. After cutting the circle the cover-glass must be wiped very carefully with a piece of soft material. A few splinters of glass are apt to remain which, getting entangled with the oil, may adhere to a homogeneous objective, and, if the front lens be wiped immediately after, may cause some scratches upon it in so doing.

6. In searching a slide to discover the whereabouts of a circle, it must be recollected to focus *the upper surface of the cover-glass and not the specimen*; and a low power ought always to be used. Especially should it be avoided to hunt for the ring with an immersion objective, as the cedar oil, filling up the engraved line with a fluid of approximately the same refractive index as the cover-glass itself, may entirely hide it from view.

A still simpler arrangement was devised some years ago by the author, and used by him very considerably. It consists in having a "dummy" objective turned in brass to fit the nose-piece. The end that would correspond to where the little hemispherical front lens is seen in ordinary, is made to taper to a blunt point a little larger than the diameter of a rubber letter "O" (such being about 2 mm. in diameter), which can be obtained from any rubber-type manufacturer. It is fixed to the brass front with seccotine. After having found the position to be recorded, the objective is removed and the "dummy" substituted. A little common ink dabbed on to the rubber "O"

makes a mark on the slide when the fine adjustment lowers it sufficiently to bring it gently in contact with the cover. The object then is found in the "O."

The Indicator Eyepiece

This useful contrivance, shown in Fig. 227, is for the purpose of indicating to an audience a special position of interest in the field of view. The actual pointer, which is sharply in focus of the eye-lens, possesses a limited amount of movement by means of the little handle to which it is attached. To use the arrangement the object, whilst being looked at through the ocular, is shifted upon the stage until the special place of interest lies within reach of the needle, which is then delicately adjusted by means of the handle above described. When several are looking through the instrument in succession, this means of indicating the special point of interest saves a great deal of explanation.

Fig. 227.
Indicator Eyepiece.

The Measuring Ocular

An approximately accurate measurement of an object may be made by employing the "Measuring Ocular." This little piece of apparatus is usually a simple Huyghenian eyepiece specially provided with a sliding eye-lens so that a divided scale—called an "eyepiece micrometer," that is dropped on to the diaphragm—can be accurately focussed. After this is carefully done, the eyepiece is dropped into the draw-tube in the ordinary way. To ascertain the value of the divisions they are compared with those of a stage-micrometer, the name for a 3 × 1 slip having a cover-glass fixed upon it that has been ruled with lines a known distance apart, placed on the stage of the microscope. Supposing the stage micrometer is divided into spaces $\frac{1}{100}$ of a millimetre, and that one of these is contained in 5 of the eyepiece micrometer, then it is obvious each of these spaces is $\frac{1}{500}$ of a millimetre. This value being arrived at by

taking several observations and employing their mean, the micrometer on the stage is removed and the object to be measured substituted, care being taken that the draw-tube is not touched whilst doing so. Several measurements should be made and their mean adopted. See Rheinbergh's inventions, Addenda.

A form of micrometer sold by Watson & Sons, called "L'Escalier Eyepiece Micrometer," consists of a ruled glass disc with a broad scale of 100 divisions and figured at every tenth division. The area of each group of ten divisions is divided diagonally into black and clear spaces, which facilitates the process of counting or measuring. It was originally suggested by Leitz.

A form of micrometer known as a "Jackson's Micrometer" has a scale in a brass mount that slides, in approximate focus with the eye-lens, across the axis of the eye-piece. Means are provided for accurately focussing this scale by the eye-lens, and a fine screw is added to facilitate the placing of the lines laterally upon the object to be measured. A very similar apparatus, called by Zeiss a "drum ocular with micrometer," has a drum to record the number of rulings actually employed. Both of these micrometers are evaluated by means of a stage-micrometer as above explained.

The Spectroscopic Ocular

This consists of a Spectroscope in conjunction with a Huyghenian eyepiece, the combination being so constructed as to be readily attachable to an ordinary microscope. Before describing how to set up and use the same, it will be convenient first to describe the general details of the arrangement.

The tube A in Fig. 228 is of the same diameter as an ordinary ocular, and drops entirely into the draw-tube of the microscope, being held firmly in position (so as to prevent the whole apparatus from swinging round) by tightening the fixing-screw B. The drum C (the interior of which is shown in Fig. 229) contains the slit and the comparison prism, the screw E regulating the length of the former, whilst F controls its width, the comparison prism being thrown in or out of use by the lever G. The mirror H reflects the light through the comparison object—placed beneath the springs I, I upon the stage S—on to the prism within the

drum. This, when brought into action by the lever G, casts the comparison spectrum into the field of view, lying immediately above the spectrum from the object on the stage of the microscope which arrives through the tube A. As seen by the observer in the ocular these two spectra in no way interfere with one another as they are not really superimposed, but merely placed one above the other in juxtaposition. By this simple and convenient arrangement the two spectra can be very readily compared.

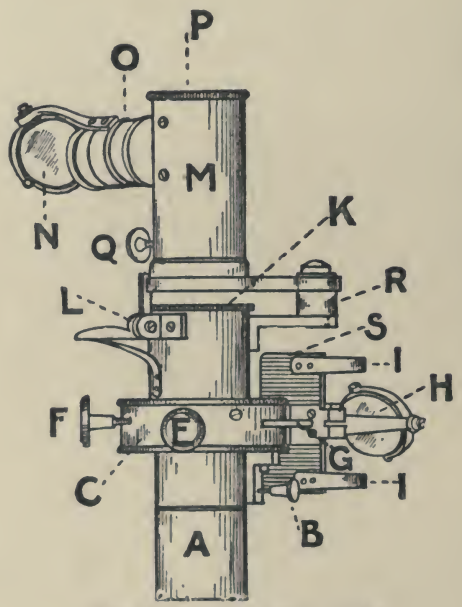


Fig. 228.—Spectroscopic Ocular.

The eye-lens of the Huyghenian ocular lies at K, and, being mounted in a separate tube, can be pulled out or pushed in to a limited extent so as accurately to focus the edges of the slit which lies—as we have already said—within the drum C.

The field-lens of the ocular is placed in a position corresponding with the black line seen crossing the tube A, at which point the tube can be separated when the lens surface requires dusting or the underside of the slit demands attention.

The catch L holds the tube M containing the spectroscopic prism *in situ*, but by pressing the end of the lever the whole of

the upper portion of the arrangement is released and can be turned aside upon the joint R.

The mirror N illuminates a wave-length scale fixed at the adjacent end of the tube O, within which is a draw-tube containing a little lens to focus the details of the scale quite sharply when viewed by the eye of the observer placed at the opening in the diaphragm P. The image of this scale being reflected off the first surface of the spectroscopic prism, the observer on looking down the hole in P sees the wave-length of the respective colours, both of the spectrum of the comparison object and that of the object upon the stage of the microscope. If it be desired to study the latter spectrum only, the comparison prism can be cast aside by merely pulling the lever G away from the stage S.

Fig. 229.— Interior of the Drum, showing the Mechanism of the Slit.

With respect to setting up the instrument, before actually doing so the slit should be cleaned, unless it is known to be free from dirt. To get at this, which is within the drum C, the lever L is pressed downwards and the upper portion of the combination swung aside. Having lifted out the tube containing the eye-lens K, the screws E and F are turned in the opposite direction to the hands of a watch, which increases both the width and the length of the slit, and the comparison prism is turned aside by pulling the little lever G away from the stage S as far as it will move. A piece of wood sharpened to a fine delicate point (a match or some of the special wood used by watchmakers for watch-cleaning will do) or a quill toothpick is now lightly

drawn along the jaws whilst the operator gives a gentle blow of the breath. After doing this, it is probable no dirt will be left. If, however, after returning the lens K and carefully focussing the slit (which should be all but closed) and replacing the spectroscopic prism to centrality notified by a click of the lever L, *black lines are seen running parallel with the edges of the jaws along their entire length*, they indicate that some foreign matter has still been left unremoved, and the operation of cleaning must be again repeated.

The apparatus is now ready to be dropped into the draw-tube of the microscope, the screw B being tightened to hold it firmly *in situ*, care being taken that the face of the stage S is directed towards the illuminant. Having turned the lever G towards the stage as far as it will go—which means the prism within the drum is brought into action—the mirror H must next be fidgeted about until it reflects a beam of light from the illuminant through the centrally placed hole in the stage S, and the position found by direct experiment, which will furnish the most brilliant spectrum possible to the eye of the observer when placed at the hole in the diaphragm P. Having accomplished this adjustment, the substage mirror of the microscope is manipulated in the same way so as to turn a beam of light into the tube of the instrument, and thence through the spectroscopic prism into the field of view. When this is done, two spectra can be seen through the ocular, the upper one having arrived through the comparison prism, and the lower one through the microscope proper. As we have said, they do not interfere with each other, as they simply lie in juxtaposition, in which situation they are conveniently placed for comparison. If they are not of equal brilliancy the mirror H or the mirror of the microscope must be manipulated until there is no difference in luminosity.

To illuminate the wave-length scale so that it shall appear projected upon both spectra is the next operation, being effected by fidgeting about the mirror N; whilst the sharpening up of the details is brought about by sliding in or out the lens in its draw-tube contained within O.

If the lines of the scale are not perpendicular to the length of the slit, the milled ring at the end of the draw-tube O adjacent to the mirror N is turned on its axis until they are in a satisfactory position.

With one exception the instrument is now ready for use. A little consideration suffices to make it understood that if the scale of wave-lengths is to be of any real service the orientation of the divisions must be properly set with respect to the different colours, for, if not, the wave-length figures, for, say, yellow light, might be appearing over the green, or *vice versa*, and so give quite an erroneous reading. To set this scale correctly the illuminant is removed, and a large spirit-lamp, which has had plenty of common table-salt dissolved in the spirit, substituted. When the mirror of the microscope reflects this light into the spectroscope, the eye of the observer placed at the opening P in the diaphragm sees now, with the slit open, a spectrum more especially luminous in the yellow. On closing the slit by unscrewing the screw F, the yellow colour becomes narrower and narrower until at length, when the jaws are all but touching, it is reduced to a brilliantly defined and exceedingly narrow line. Presuming the scale is illuminated properly by its mirror N, the microscopist can, by turning Q, adjust the figures in such a manner that the graduation 59 lies directly coincident with the yellow line (or D line, as it is scientifically called) in question; and should the 59 line of the scale be not absolutely coincident with the yellow line *its entire length*, the milled end of the draw-tube within O should be turned until it is made so. The scale is now properly placed, and the wave-lengths read correctly if the maker has made the graduations properly. It is possible here for two pitfalls to occur. The yellow line may not appear sharp—this requires the lens at K to be raised or lowered a trifle; or perhaps the D (or "Sodium line," as it is also called) may be too faint—this requires a stronger light. If it be impossible to get a larger lamp, the whole arrangement should be removed from the microscope and pointed directly at the spirit-lamp. In most cases this remedies the trouble so far as relates to the brilliancy of the sodium line, but it should be borne in mind that to make the adjustments mentioned is far more difficult in this way, because it is not easy to keep the apparatus steady enough to illuminate the scale, alter its adjustments, and to keep a brilliant D line simultaneously.

The micro-spectroscope is used for two purposes, to ascertain the absorption bands of liquids and the spectrum displayed by other bodies. In the first instance the fluid is placed in a cell

on the stage of the microscope, a deep one if the fluid be very attenuated, and a shallow one if concentrated. An inch may be used on the microscope, but it does not make much difference what power is employed, for there is no focussing to take place. If the spectrum presents bands resembling those shown by some other fluid, that fluid may be put in a tube within the clips I, I, and its spectrum at once compared by using the comparison prism.

In the second instance, if a solid be the object under consideration, it is placed on the stage of the microscope and an objective used that will magnify it sufficiently to fill the slit as nearly as possible, the jaws being closed to exclude all extraneous light by turning F on the one hand, whilst the length is reduced if necessary by manipulating E on the other. *The exclusion of all light save that from the object under examination is, of course, highly important.* It is well not to employ a higher power objective than necessary, as such is apt to lessen the brilliancy of the spectrum.

The Revolving Nose-piece

In another part of this work objective-changers have been spoken of, but no particular mention has been made of the "revolving nose-piece." Although much employed, we have never been enthusiastic supporters of its use, excepting perhaps for changing low powers, because there exists no means of centring the objectives in such a manner that when they follow one another *the particular part of the field in view with the first shall be in the field of view of the second or third.* It is true the revolving nose-piece as now made is very perfect, but no accuracy in the arrangement itself can get over slight variations in the centring of individual objectives unless some special contrivance is present, and this is absent in all forms we have ever seen. We must say, although it may be contrary to the opinion of some microscopists, we greatly prefer to use the Zeiss "changers." Another advantage gained by doing so must not be overlooked, namely, the much less weight on the tube to that which obtains when the rotating objective changer charged with perhaps three objectives is attached to the nose-piece. The saving of wear and tear on the adjustments, both coarse and fine, must be very sensible. Whichever is employed, due allowance

must be made for the increase of the mechanical tube-length by shortening the draw-tube the correct amount.

The Van Heurck Transformer

This auxiliary lens was suggested to change a short-tube objective into a long-tube combination, or *vice versa*, a negative form being added for the first purpose and a positive one for the second. Experiments conducted by Commander Ainslie appear to verify Van Heurck's original suggestion that the best focal length to employ for these purposes was 500 mm. Uncorrected lenses were employed by Commander Ainslie, bi-convex or bi-concaves of simple crown glass. Achromatic combinations were substituted by Van Heurck, who finally stated "nothing was lost by their use." Ainslie remarks that certainly the uncorrected form does not suit the 12 mm. apochromatic, as chromatic effects are produced, possibly due to the large back lens of the apochromatic utilising the edge of the transformer.

In our own experiments with these uncorrected units employed upon several kinds of objectives, apochromatic and semi-apochromatic, dry and immersion systems, we found that combinations over N.A. 1·30 exhibited a pronounced haze over the entire field with loss of superlative definition, both being especially apparent with apochromatics, although more evident in some instances than others. Dry lenses did not appear to suffer in most cases, and definition was actually improved in one instance. What would happen with achromatised transformers we cannot say, but evidently much depends upon the individual objective.

A Simplified Method of preparing Metallurgical Specimens suggested by Mr. W. A. R. Aird

The difficulty in preparing metallurgical specimens is too well known to refute. The following simple means can be relied upon as being both practical and effectual.

Four to twelve sections are cut off a $\frac{3}{16}$ rod of the material

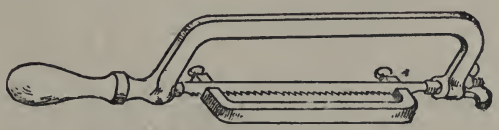


Fig. 230.—Hack-saw with gauge.

under examination, by means of a hack-saw provided with a gauge, to ensure all being of the same thickness (see Fig. 230).

Figs. 231 & 232. Specimens before and after filing oblique surface.

Each is then placed in a vice whilst a small flat surface is filed obliquely to the axis of the cylinder on one side—as will be readily understood by consulting Figs. 231 and 232—which is for the set-screw of the holder to engage upon and so prevent the specimen moving, whilst undergoing the processes of grinding and polishing.

The sections are now placed in the holder above alluded to and shown in Fig. 233—which consists simply of a plate of phosphor bronze containing twelve circular recesses, each of which is provided with a set-screw—and the tops filed flat. The screws released, the little cylindrical pieces of metal are now removed by means of the handled rod (Fig. 234), which is pushed through the holder from its under side in a manner illustrated in the same figure, and subjected to any treatment that is under investigation.

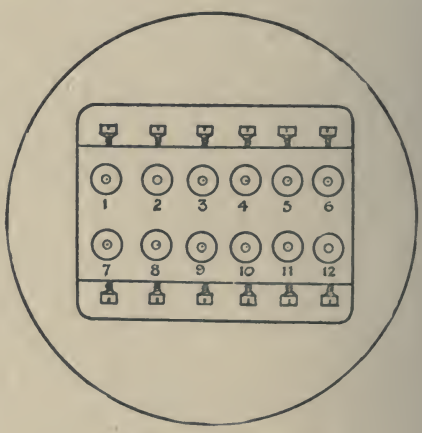


Fig. 233.—The Holder.

Replacing and refixing them in the holder, they have now to be ground and polished. The former operation is carried out by means of a piece of carborundum 3 × 3 × ½ in. in size; whilst the polishing—taking from half to three-quarters of an hour—is effected by means of the rubber (Fig. 235), which is covered successively with six grades of emery paper, Nos. 3, 2, 1, 0, 00, 000, the actual final surface being obtained by using rouge or diamantine on the parchment that covers the block shown in Fig. 236. It is easily understood that all the twelve specimens can be treated at one and the same time, and that moreover the holder

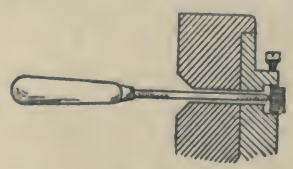


Fig. 234. Handle removing specimen.

can be placed under the microscope for examining any one of the sections at any moment without its removal from the block, which is a matter of great convenience, and one that saves a great deal of time and trouble.

Fig. 235.
Rubber for emery paper.

When the polishing is considered satisfactory, each specimen is loosed from the holder and held in the steel forceps (Fig. 237) whilst undergoing immersion in the tube of etching fluid, as shown in Fig. 238.

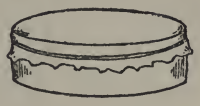


Fig. 236.—Parchment covered rubber.

Fig. 237.
Steel forceps with specimen.

By tilting the forceps when in this position, the observer can examine the specimen—even with a hand-lens—from time to time to see how far the etching has progressed, without its removal from the tube, which is an example of the advantage gained by the suggestion of Mr. Aird to use a *tube* instead of a dish, more commonly employed for this particular part of the process. When the chemical action has sufficiently progressed, the forceps and specimen are lifted out of the fluid and dropped into another tube—one of those shown at the back of the rack (Fig. 239)—which before commencing operations has been filled with some neutralising fluid such as lime-water, when all further chemical action immediately ceases. At the front of the rack—held in position by the little springs, only one of which is shown in the figure—is a strip of paper upon which it is intended the experimenter shall write from time to time, for future reference, details of the treatment to which each individual cylinder of metal has been subjected.

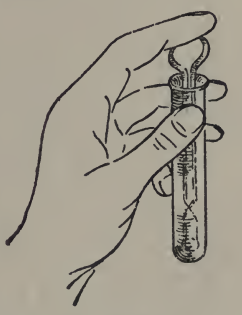


Fig. 238.—Etching tube.

It should be mentioned that the great advantage of having the specimens of one definite thickness is now very apparent, for after once adjusting the vertical illuminating apparatus to an ordinary microscope (generally a somewhat tedious and lengthy proceeding), all the samples may be quickly examined and compared *without any re-*

adjustment of the light, which means a great saving of time and trouble. If desired they may be loosely arranged on a slip provided with a ledge, which allows any two specimens to be brought into the field at one time, so facilitating comparison; or a series may be passed under the objective in any order that may be considered desirable. By this simple arrangement, too, the microscope can be *inclined* without affecting the examination in any way. Altogether the whole arrangement, both for simplicity of method and for the cheapness of the apparatus employed, betokens the voice of authority as well as that of one thoroughly acquainted with all ordinary methods and their attendant troubles.

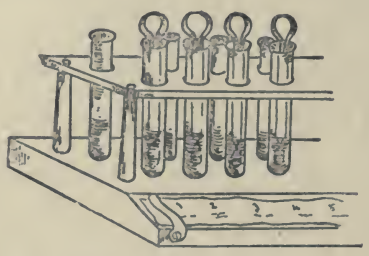


Fig. 239.—Table rack.

Wright's Eikonometer

This instrument has been invented for obtaining the magnifying power of the microscope otherwise than by the usually adopted methods previously described. It consists of a piece of apparatus not unlike an eyepiece, which is held over the ocular of the microscope without in any way interfering with any of the adjustments of the instrument, and the object can be instantaneously measured. When it is used the object being examined is seen just as if the eikonometer were not in use, and a scale of divisions representing millimetres is seen superimposed upon the object. This shows at once how many millimetres the picture of the object measures. To obtain its actual size this measurement is divided by the magnifying power, which is obtained by means of the eikonometer as follows, and may be kept for reference on a card in the eikonometer box.

Place a stage micrometer on the stage of the microscope, and hold the eikonometer over the eyepiece; the eikonometer divisions, which represent millimetres, will be seen over the stage micrometer divisions which represent one-tenth or one-hundredth of a millimetre; thus, if the hundredth of a millimetre of the stage micrometer measures five divisions of the eikonometer, the

magnifying power is 500. This having been noted, all objects viewed with this object-glass and eyepiece and the same length of draw-tube can be instantly measured. If on using the eikonometer the object under observation is found to measure one millimetre, its actual size is one five-hundredth of a millimetre; if two, two five-hundredths of a millimetre, and so forth.

It may be interesting to add an explanation of the principle of the eikonometer. See Fig. 240.

If A and B represent the object-glass and eyepiece of the microscope, it is well known that the image of the object at D

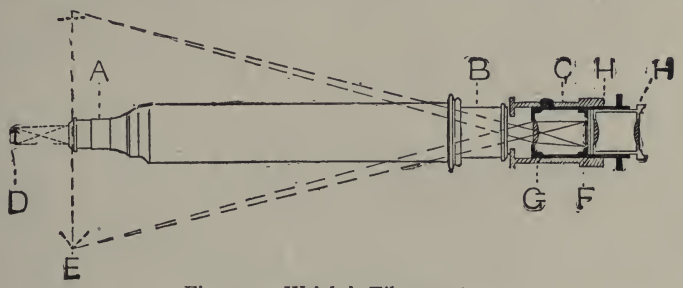


Fig. 240.—Wright's Eikonometer.

is represented by the microscope as a virtual image apparently existing at E, and the eye placed over the eyepiece sees the picture as if it actually existed at E at a distance of ten inches (the visual near point). The eikonometer C, when placed on the eyepiece, produces at F an image of the image at E, one-tenth the size, and a micrometer scale of tenths of a millimetre is placed at this position, the eyepiece H being a convenient magnifying power with which to examine the picture at F. The eikonometer therefore is a means of measuring the image which is formed by the microscope at E.

CHAPTER XVIII

HINTS

Upon Several Common Faults met with in using the Microscope and its Accessories

Their Cause and Means of Removal

1. If when using a low power the light be found too powerful.	1. Lowering the condenser a *little* may ease the intensity of the illumination; but if this be not sufficient a piece of very thin black or opal or ground glass may be placed between the mirror and the source of light. A monochromatic screen, such as a Gifford's F-line filter or a piece of the special green glass as recommended by the author, may be tried.
2. If the illuminant be too faint.	2. This may occur when the substage condenser is not used, or if employed is of smaller N.A. than the objective. Sometimes it arises from omitting to use critical light. With very high-power objectives and high oculars, when the field is too dark, there is no alternative but to use a stronger illuminant. We have not often—save for dark-ground illumination with high powers—wanted a more powerful electric lamp than the cheapest form of Nernst, which is convenient, as it can be attached to the ordinary house circuit: but, if a still more powerful illuminant be required, we know no better one than the new miniature arc-lamp by Leitz described in the chapter devoted to "Illuminants."

3. If when using an oil condenser the fluid runs over the stage between its slides, making them all work far too stiffly.	3. The stage and its slides must be cleaned with xylol. Let it be freely used and the parts carefully wiped. If then they work stiffly use a very small quantity of *good* clock oil, such as that sold at most of the tool-shops, more particularly those dealing with watchmakers' requirements, such as Cooper's in Hatton Garden, London. After oiling, wipe nearly dry with a thoroughly clean cloth.
4. If when using a $\frac{1}{12}$th or any immersion objective, just after "taking to the slide," the field of view, as seen looking down the ocular, becomes quite dark with a kind of wave running across it, entirely obliterating the illumination or spoiling the definition.	4. This comes from a bubble of air in the oil on the objective, or on the cover. Sometimes after lifting the objective back again off the slide and returning to the cover this again recurs. Under these circumstances wipe both objective and cover clean, and start again with fresh cedar oil. If changers be used, don't raise the objective, but merely slide it off and then return it. This nearly always cures the trouble. If the bubble be very persistent, the cover and objective had better be cleaned with xylol.
5. If whilst using an immersion objective with oiled condenser the field suddenly loses a great deal of light and the specimen becomes almost totally deprived of fine definition, no distinct wave of darkness being first noticed.	5. The oil has left the slip *between it and the condenser*. This usually arises when the slip is rather *thin*, and the condenser is made to be used with a thick one. Take a cover-glass and place it between the top lens of the illuminator and the underside of the slip, *oiling both sides of it*. If the trouble recurs, add another cover or use one twice as thick.
6. Occasionally when setting the mirror, the light being in a particular position, the whole of the back lens refuses to be illuminated; no adjustment seems to be of any avail, and yet the oiling is complete.	6. The fault lies in the angle the microscope is bent at, with relation to the position of the illuminant. The portion that *will not* illuminate is the edge of the iris-holder. This can be proved by putting the fingers in the position, when they will be seen on looking at the back lens of the objective. Raise or lower the light, or alter the

angle of the instrument. It mostly occurs when the microscope is too much inclined.

7. If definition of some well-known specimen fails to appear as good as usual apparently from no cause.

7. This may arise from several faults. The light may not be truly critical; the condenser may not be the correct one with respect to aperture. The iris may have become accidentally closed. The objective, ocular, or illuminator may have turned foggy, this accident not unfrequently happening with some kinds of the Jena glass. Take off each of these and look at them carefully with a rather feeble light, turning them a little edgeways and holding them some little distance from the eye. They must be sent to the manufacturer if faulty; don't attempt unscrewing. Sometimes in cold weather the ocular may steam: this wipes off easily. The oil may have left the condenser or the cover. Some dirt may have fallen on to the back of the objective, or a piece of thick fluff. Remove with camel's-hair brush. *Be careful not to scrape or injure the back lens* by rough usage when so doing. Occasionally the medium in which the specimen lies may have become opaque. If using a dry lens a spot of oil may have accidentally fallen on its front lens, or perhaps on the cover-glass. Look at the objective, and if oil be there use xylol, avoiding spirit of any kind to clean it with. Wipe the cover with the same fluid. The objective may be working at the wrong tube-length, or the iris may be too wide open, and the specimen is being flooded with light. This usually only happens if the illuminator is of greater aperture than the objective, unless the latter have a very poor outer zone. Sometimes when a bull's-eye is being used, it may be out of its best position or be placed edgeways—that is, the axis of the auxiliary condenser may

not be in line with the mirror and the illuminator.

8. The object will persist in rapidly going out of focus after being carefully set.

8. This may arise if the microscope be slightly inclined or horizontal from the fine adjustment having become stiff from non-use of the instrument for some time, or from the lubricating oil having become dried up. As an immediate remedy, but only one of a palliative nature, tap fine adjustment with the finger several times and run the fine screw up and down very quickly and freely—the specimen being removed out of "harm's way." As a curative treatment the adjustment must be taken to pieces, cleaned, and fresh oil or other lubricant be used. If the microscopist be not a mechanic, it is best to return the instrument to the maker. If the microscope be horizontally placed, as in using direct light, the above trouble may be the cause; but in addition it may arise from the cover (in a newly made specimen) slowly sliding down the slip. If time cannot be spared to allow of it drying properly, put a dab of vaseline on the lower edge. It will usually hold it *pro tem.* Sometimes this occurs from the slip not resting firmly on the stage although oiled to the condenser, resting more on the front lens of the illuminator than on the stage. Lower the condenser and re-obtain critical light. The fault may occur from the objective working *just on* the cover because the latter is too thick. The latter then bends with the fall of the objective and keeps bending for a while. Instantly raise objective, and don't attempt to use it.

9. If the object keeps floating out of focus and *up* the field of view.

9. This arises from a too newly made specimen—remedy as above in No. 8. It may arise from a specimen actually moving in a fluid medium

	down between the slip and the cover. Use the microscope vertically. It usually cures the trouble directly. The rack of the stage may be loose and so allow the whole stage to travel downwards.
10. If when using an aperture over N.A. 1·0, and when oiling to the slip has been carefully carried out with all precautions, the back lens *will not completely fill with light*.	10. This arises from the object being mounted in air, and so only transmitting approximately an N.A. 1·0 cone. No remedy save remounting properly. This is often seen when using a dry mounted diatom in mistake for one in a highly refractive medium.
11. If bacteria or like objects appear to have a white capsule around them.	11. The iris is too much shut down, or the condenser is of too small an N.A. for the objective.
12. If when using a *very* high-power ocular, and condenser properly oiled, the object (an extremely minute one) looks fuzzy and ill-defined—more so than it should.	12. This may arise from the illuminating cone being too small, the iris having been too much cut down, hence the circle of confusion becomes larger than $\frac{1}{100}$th of an inch. Open iris to the full. If no improvement, use monochromatic light; green may suffice, but employ blue if possible. See that the front of the objective and that of the condenser, if immersion systems, are clean and free from partially dried oil.
13. If immersion objectives have been put away for some little time unwiped, their definition appears sometimes materially injured.	13. The immersion oil has become dried on to the minute front lens, having sunk into the little rebate between the edge of the mount and the glass front itself. This takes some time to dissolve in fresh oil. Clean very gently with a soft piece of cambric and xylol. Avoid using spirits of wine or anything else. Never use a piece of stick to clean the lens front with—as sometimes suggested—simply a moistened piece of cambric.
14. Using a dry lens or a water immersion after having employed an oil immersion, the object looks ill-defined.	14. This often arises from a thin layer of oil being left on the cover-glass. Clean with xylol. Don't forget to regulate the aperture of the iris to

	meet the change of N.A. between the objectives.
15. If when looking at a diatom, the light seems cast sideways, distorting the object.	15. The mirror is not correctly set, or the condenser is extremely out of centre with respect to the optical axis. The specimen maynot be mounted flat.
16. If when using extreme oblique light and a high-power oil immersion, colours—rainbow effects—are seen.	16. The condenser is not achromatic.
17. If specks appear in the field which *move* with the eyepiece.	17. The ocular is dirty ; clean with a small quantity of spirits of wine used with a piece of cambric. Don't swab the lenses, but merely firmly wipe them with the moistened fabric. Separate the ocular if necessary.
18. If above appear *stationary* when the eyepiece is rotated, although they seem to float about the field of view.	18. The observer's eye has specks in the vitreous humour. Lift the eyes up and down violently, and roll the head from side to side. They will often be cast out of the way. They disappear in a few hours, but occasionally take some days—the observer's health not being in good order may often be the cause.
19. If the draw-tube becomes too stiff for easily adjusting to the correct tube-length of an objective.	19. It must be removed ; wiped quite clean with xylol and its sleeve likewise. Oil both well with the oil previously mentioned (see No. 3), or some other non-acid preparation, and then wipe both *very fairly dry*. Fit together, separate, and *again wipe*. This treatment must not be used with a cloth-lined draw-tube.
20. If racks run stiffly.	20. Lubricate with the above-mentioned oil and wipe nearly dry. Should the teeth be dirty, clean with a match-stick first before oiling. Occasionally, when an instrument is not made accurately, the rack is filled with stiff grease to prevent "backlash." After removal, fresh must be used. Clarified lard thickened with white wax answers very well.

21. If substage runs stiffly.	21. Clean and oil as before mentioned.
22. If oculars drop stiffly into the draw-tube.	22. Clean draw-tube and ocular mount with xylol, oil and wipe clean as in No. 19.
23. If with the Continental form of illuminating apparatus the iris suddenly appears out of centrality, or, what is the same thing, the condenser suddenly appears eccentric.	23. This arises from the iris slipping out of centrality, the clicking arrangement having worn so loose that when turning it to arrange for oblique light, it has become detached. Bend the clicking-spring to make it hold tighter.
24. If when using illumination for an opaque object, as the tube is lowered to accomplish accurate focussing, the object becomes very dull and badly illuminated.	24. It is because the mount of the objective has got between the light and the object as the tube was lowered. Place the light more obliquely, so that the rays do not strike the objective.
25. If when using the polariscope the colours do not appear as bright and vivid as they should.	25. This arises from one or two causes. The lower nicol (the polariser) may not be placed at its best position with regard to the mirror. Remove selenite, quarter wave plate, and specimen, and keep rotating the *polariser* until, when turning the analyser, the best black and white effects are produced. Then find the best position for "maximum effect" with the selenite, and after that the same for the quarter wave plate if employing circular polarisation. If the colours are not as expected, differing from those obtained with other objectives, the objective furnishing the faulty appearance may itself be faulty. Take it off the microscope and place between two nicols. On revolving one or other *no colours* should appear. If they *do*, return to the maker.
26. If when using polariscope the field is unequally illuminated.	26. This is because the condenser does not suit objective, the mirror is not properly placed, or the concave side is not turned (or *vice versâ*, the

	flat side) to the condenser. It may arise from using a very low power, and the polariser not being sufficiently *large* to cover the field.
27. If when measuring the size of an object with bifilar micrometer the threads appear blurred when the focus of the object is perfect.	27. This arises from the webs not being properly focussed with the eye-lens *before* looking at the object. Adjust the eye-lens to make them so and re-focus.
28. If with very high magnification the object remains stationary or fairly so, but the wires appear to shake so much that measurements cannot be made with a bifilar micrometer.	28. Because of the "shake" of the whole apparatus. Try using Mr. Nelson's suggestion for supporting the micrometer on a stand *separately* from the microscope in this class of work.
29. If the Nernst lamp won't "light up" when the current is turned on.	29. Because the plug is put in the wrong way for "the poles," "the furnace" connection is broken, or the light filament is fractured. If the furnace is wrong a spirit-lamp may be applied to the filament whilst the current is on. The heat will cause the current to pass in a few moments, and the light will then appear. If the filament is broken a new burner must be obtained.
30. If the ordinary microscope oil-lamp will not burn brightly.	30. The wick may want wiping with a cloth to remove the charred portions, the oil is bad, or the vent-holes around the burner are choked. Remember, that to get the best light and the freest from smell, to turn up the lighted wick till it smokes, and then to turn *down* until it just ceases to do so.
31. If with the ordinary lamp the flame image is too small with a low power to include or cover the object, and yet the bull's-eye makes the light too strong.	31. Try turning the wick broad-way on, *after obtaining critical light* with it edgeways.

32. When using dark-ground illumination with a wheel diaphragm or a Wenham's paraboloid, curious-looking streaks or ill-defined patches of light are seen scattered over the field.

32. These are due to a dirty cover-glass. If wiping carefully does not remove them they are on the *inside* of the cover. This shows the specimen is mounted "dry," and that dirt has either been left on the cover or it has somehow got there after mounting. Nothing but cleaning this surface will remove the objectionable particles.

33. When attempting to centre a condenser, it may be found that the iris *cannot be focussed* even with a low-power objective.

33. This is a serious structural defect not infrequently found in Continental microscopes of older types (so in selecting a second-hand instrument should be looked for previous to making a purchase), and arises from the position of the iris with respect to the condenser being incorrect ; usually too close. This not uncommonly occurs when the optical parts of the illuminator are made to fit into the sleeve from below upwards. To remedy the defect it is best to have the condenser made to fit into its sleeve from above downwards, *taking care it does not drop in too far*. By thus increasing its distance from the iris this plan usually remedies the trouble. As an alternative the condenser may be arranged to fit into a "centring adapter," such a one as made by Messrs. R. & J. Beck, described on p. 45, which has the same effect. If this be not found satisfactory there is no alternative but to have a centring-tube made to drop into the iris fitting so that its end protrudes about half an inch below the leaves of the iris towards the mirror. Provided this tube be made to fit *accurately*, and its end is perforated at its *exact centre* (corresponding with the axis of the tube and so with the optical axis of the instrument) by a *very* small hole, this minute aperture can hen be used instead of the closed iris, and in a similar manner, for the purpose of

centring the condenser. If however any difficulty arises it is best to consult an optician. Perhaps the iris does not close small enough for the purpose in question.

34. When using a binocular the image looks ill-defined.

34. This may arise from several causes. Focus the object with the left eye to the left tube, then look into the right tube with the right eye. Focus should be similar (with normal vision). If not, try and make so by altering the draw-tube of eyepiece. If none exists, try altering the depth of the eyepieces themselves in the tubes. If no improvement, change oculars ; perhaps they are not in their proper tubes. If no improvement, remove the prism box with its prism. Clean prism and return with box into microscope. See if improvement results by not quite "pushing home" the box. If still no improvement, lift out prism with its cell from the box and note position of prism in cell. Try small alteration in position of prism ; perhaps it is not set exactly to your best definition. If improved by any slight adjustment, fix by dropping a small quantity of gum between prism and its cell or return to maker for adjustment. The plane end of prism should face at right angles to the axis of the lens.

35. When the front lens of an oil condenser or of an oil objective becomes fixed with oil that has dried.

35. No pincers or other instrument should be used if it can be possibly helped, as injury is nearly always likely to occur. The parts will frequently separate if the line of union be liberally painted with xylol and allowed to remain for several hours. The union line should then be carefully cleaned and the painting done over again, leaving all at rest, say, the whole of the night. In the morning the parts will usually separate by the use of the fingers only.

CONCERNING THE PLATES

As a test-object for showing the separating and defining power of an objective is especially chosen on account of its difficulty of resolution, it can be readily understood that to reproduce its details by photography, even to approximate perfection, taxes the skill of the photomicrographer to the utmost. Hence the reader when criticising the plates with respect to the performance of the various objectives should be distinctly warned not to regard the limit of perfection displayed by the author's photomicrographs as the true limit of perfection or excellence capable of being witnessed when the objectives are *visually used*, as due allowance must always be made for a certain amount of falling off in this respect, which seems inevitable and unavoidable with any known method of reproduction.

The negatives of the first two editions were taken on Edwards's Isochromatic Plates, the Barnet Plates, or the "Flashlight" of the Imperial Company, the last being employed for Amphipleura pellucida in "dots" on account of the extreme sensitiveness of the emulsion. In the third edition, the new negatives were made with Ilford Medium and Rapid Chromatic, as well as upon the Barnet and Imperial manufactures.

Seeing that when monochromatic illumination is used exposures of some sensible duration are needed, the strongest limelight was necessary. This entailed the employment of a very powerful mixed jet, one especially made by the well-known firm of Beard, concerning the performance of which we feel justified in bestowing the highest praise. It was essential also that the oxygen used should be as pure as it is possible to obtain (otherwise the illumination would have been considerably reduced in intensity), and we feel we ought to mention the excellence of that procurable from the British Oxygen Company

(formerly Brin & Co.), Westminster, which we have reason to know positively, from very frequent testings, never contains impurities more than the almost vanishing point of 5 per cent.

As the jet was so powerful—using about ten feet an hour of coal gas as well as oxygen—it was necessary to employ the best hard Nottingham limes procurable to prevent as much as possible their falling to pieces or cracking during an exposure.

On certain occasions, such as the photographing of Amphipleura pellucida, a 30-ampère electric lamp by Zeiss was employed—notably with Figs. 5 and 6, Plate XIII., and Fig. 2, Plate XV. The photographs in this third edition were all taken with some form of electric light, the little arc described on page 167 being often found extremely useful.

The printing from off the negatives for the first and second editions was almost entirely executed by one of the daughters of the author, "Nikko" or the P.O.P. of the Imperial Company being employed. The new photographs of the third issue were made on the same material and sometimes on the shiny paper of the Criterion Company by the author himself, as his daughter was pre-occupied by honorary war work.

With respect to the actual reproductions, the author has already in the Preface to the first and second editions expressed his indebtedness to Mr. Alfred Dent for his care and personal supervision in the manufacture of the blocks, so that all particulars shown in the originals should be accurately represented. In this the third issue, owing to unforeseen difficulties (war), Mr. Walker was asked to reproduce the new blocks in the place of Mr. Dent. The author is very desirous to thank him for the great trouble and unceasing pains he has taken to carry out the same ideals of perfection which were aimed at by his predecessor.

In addition the author is further desirous of acknowledging his indebtedness to Messrs. Hazell, Watson & Viney and their executive for the care and trouble they have taken in *printing* the "Plates" so as to obtain as much from the blocks as possible; as well as to Mr. John Murray for sparing no expense incurred in this matter. This remark applies to the third as well as the two previous editions of MICROSCOPY.

APPENDIX

THE RELATIONS BETWEEN OBJECT AND IMAGE

The terms Image Point, Equivalent Focal Length, Principal and Cardinal Planes explained

WITHIN certain limits lenses or lens systems can produce images at any point on their optical axis; the purpose of this note is to show how the size and position (*i.e.* whether erect or inverted) of this image and the corresponding position of the object may be most easily ascertained. In so doing various terms employed in this book are explained as they come into use.

The underlying principle is derived from the consideration that within the limits in which a lens or system of lenses can be successfully used, *i.e.* within which it gives sufficiently sharp images, all rays entering the lens system from any point in the object are with sufficient precision reunited in the corresponding, or conjugate, point of the object. From this it necessarily follows that if we can trace one single ray from the object point through the lens system, we already have a geometrical locus for the image: it must lie somewhere on this ray. And if we can trace a second ray, differing from the first, then the image point must also lie on this second ray; hence its position is now definitely fixed at the point of intersection of the two selected rays.

Any two rays might be chosen, but it is found by far the simplest way to determine the course of parallel rays entering the lens system from either side and to select two rays out of these two bundles of parallel rays, which pass through any object point in order to determine the position of the image point.

In Fig. 241[1] let the space enclosed between LL represent any lens or system of lenses whatever. Let it be known from experiment that a ray parallel to the optical axis—and near to it—such as the ray 1, when emerging from the lens proceeds in the direction F_1—1. Produce the incident and the refracted ray until they intersect at Q_1; then it is evident that if a very thin lens were placed in the plane P_1P_1 which contains Q_1, it would refract the ray 1 so as to have the same final

position and direction as F_1—1, provided that the assumed *thin* lens had its principal focus at F_1. Hence we may say that the lens system acts on parallel light incident from the left side exactly in the same way as would a thin lens placed at P_1P_1 and having its principal focus at F_1 The position P_1P_1 of the equivalent thin lens is known by the name of **The Principal** or **Cardinal Plane**, and the focal length C_1F_1 of the equivalent lens is described as **The Equivalent Focal Length** of the lens system.

It will be seen at once that we have already solved half the problem : for let an object of certain size, such as the arrow in the extreme left of the figure, be given, and the size of its image be required to be determined; then as the point of the arrow lies on the ray 1—Q_1, its image must be *somewhere* on the refracted ray Q_1F_1 or its continuation. As stated above, this ray is the geometrical locus for the image of the arrow point. By placing the arrow at a suitable distance from the lens system

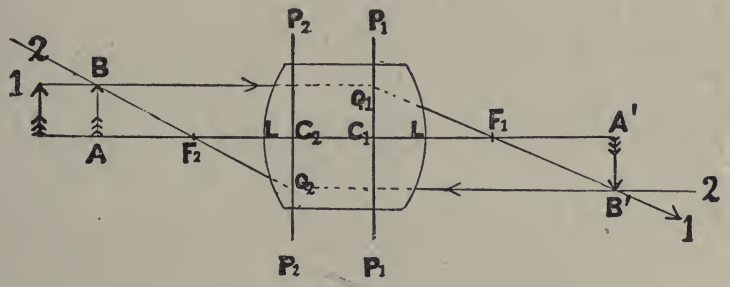


Fig. 241.[1]—The Relations between Object and Image.

it is possible to produce its image at practically any point. Supposing for example we thus produce a sharp image at A^1B^1, then we at once have the means for determining the size of the image in this position : C_1Q_1 is obviously equal to the object arrow, and C_1F_1 has been already determined as the equivalent focal length of the lens system. The triangles $Q_1C_1F_1$ and $B^1A^1F_1$ are similar; hence we have the equation or proportion—

$$\frac{Q_1C_1}{C_1F_1} = \frac{A^1B^1}{A^1F},$$

$$\text{or, transposed, } \frac{A^1B^1}{Q_1C_1} = \frac{\text{Image}}{\text{Object}} = \textbf{Magnification} = \frac{A^1F_1}{C_1F_1};$$

in words : *The magnification is found by dividing the distance from the focal plane to the image by the equivalent focal length of the lens system.* This is of course the rule which has been made use of in the text of

this book for the opposite purpose of determining the Equivalent Focal Length and the position of the Focal Plane (page 125 *et seq.*).[1]

To complete the subject, however, it is only necessary to repeat the process already employed *in the opposite direction—i.e.* to next study a parallel ray passing from right to left through the point B^1 of the image of the arrow. In the same way as before a second Principal or Cardinal Plane P_2P_2 and a second Principal Focus F_2 are found, with a corresponding Equivalent Focal Length C_2F_2. We again reason that the ray Q_2F_2 is the locus for the point of the object arrow; hence the latter can only produce a sharp image at A^1B^1 if placed at AB, where the rays 1 and 2 intersect.

For the present purpose this part of the problem is of less importance: it is settled automatically by focussing. It is necessary, however, to point out that a somewhat difficult theoretical investigation shows that the two equivalent focal lengths (the upper and the lower) of *any* lens system are equal if they are both formed in the same medium (air), whilst if they are formed in different media, the two equivalent focal lengths are in the *same proportion as the refractive indices of the media.*

[1] Putting this another way, magnification may be said to be equal to the optical tube-length (the distance from the upper focal plane of the objective to the sharp primary image) divided by the equivalent focal length of the combination in use. Hence, as the latter quantity is the denominator, the *larger* it is, the *less* the magnification on any given tube-length. It is evident, therefore, the magnification changes in *in*verse proportion with the equivalent focal length of the objective, as shown in the following two figures, 241[2] and 241[3].

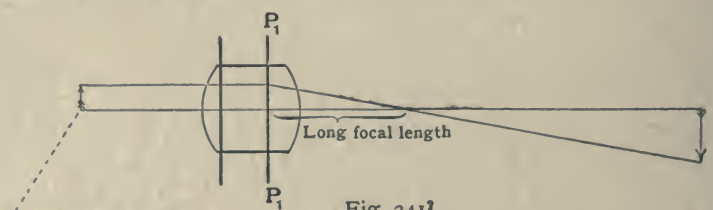


Fig. 241[2]

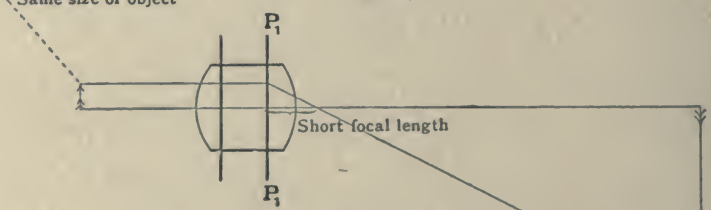


Fig. 241[3]

Thus an oil-immersion objective working in air on the side of the eyepiece and having there an equivalent focal length of say 2 mm., has an equivalent focal length of 3 mm. on the side of the object, owing to the index 1·5 approximately, of the immersion oil.

How to ascertain the Area of the Field of View with any given Objective used with any special Ocular

For particular purposes—more especially perhaps connected with making a blood-count—it may be of considerable utility to be able to discover the exact area of the field of view when a special objective is used with a particular ocular.

Although some opticians furnish the approximate diameter of the field when a particular objective is employed with some specified eyepiece, none that we are aware of ever explain how such figures are arrived at or how the dimensions given can be converted into area, so that the entire grasp, so to speak, of the field of view can be ascertained on any special occasion.

Owing to the fact that the aperture of the diaphragms in eyepieces is not, or may not be, of precisely the same size to make the figures trustworthy or anything other than an approximation, it is necessary for scientific accuracy that *each* combination of objective and ocular be separately measured; for it does not necessarily follow because *one* twelfth and ocular furnish a certain area with prescribed limits, that the same figures would be absolutely correct if used with *other specimens* of objectives and eyepieces, although perhaps of the same focal length and by the same manufacturer.

To carry out the examination is very simple, all required being a Stage Micrometer ruled on glass in hundredths of a millimetre (·01).[1]

The Objective—say, for example, a 2-mm. apochromat—and a × 4 compensating ocular are focussed on the divisions of the micrometer, and the number of ·01 units, with proportional parts of the same capable of being seen, are noted. Say, for example, these figures are 21·3. This represents (when multiplied by ·01) the actual *diameter* of ·213 mm. To convert this into the dimension of area the well-known formula must be used: $\frac{\pi}{4}d^2$, where π may be said, with sufficient accuracy, to be 3·141, and d^2 the diameter just obtained multiplied by itself.

Hence $\frac{\pi}{4}d^2$ becomes $\frac{3{\cdot}141}{4} \times ({\cdot}213)^2 = {\cdot}785 \times {\cdot}045 = $ **·035 square mm.**

[1] These are made by the filmless photographic method by Rheinberg & Co., 57-60, Holborn Viaduct, E.C.1, and can be obtained at almost any opticians. Messrs. Angus & Co. usually keep a stock of different kinds to suit most requirements of the microscopist.

Seeing that $\frac{\pi}{4}$ is a constant quantity then, the rule becomes very simple. Ascertain the apparent diameter of the field with the micrometer and multiply by ·01. This result has then to be multiplied by itself, and finally by ·785, which furnishes the area displayed by the objective in question when used with the particular ocular. It should be recollected that a note be taken of the length of draw-tube employed, as it is imperative on future occasions the same shall be used; and that when using a dry objective (as most likely would be the case when dealing with a blood-count) if the cover-glass of the specimen demands otherwise than the ordinary adjustment of the draw-tube, a re-determination of the area must be made if accurate results are required.

How to ascertain the Working-Distance of a given Objective

Focus the objective upon a well-defined object placed between a slip and a cover-glass of ·17 or a ·18 mm. thickness. Cut a few strips of highly polished note-paper, and slip one between the objective and the cover-glass, taking care not to scratch the front lens in so doing. Then follow with other pieces of paper, as many, in fact, as will pass *between the strip and the cover-glass* (*not* between the strip and the objective). Raise the objective and place the strips in question between the jaws of a Zeiss cover-glass gauge or a Ciceri Smith or other micrometer, when their combined thickness will represent the working-distance of the objective required.

ADDENDA

New Binocular Arrangements

We have spoken about the binocular microscope in Chapter XI., page 253, and of the advantage especially held by some microscopists of seeing the object simultaneously with both eyes—simple binocular vision. In addition, we have mentioned the effect produced by certain arrangements which give to the image a curious sense of depth, called the "stereoscopic effect," and, further still, yet another arrangement, by which the depth-effect sensation is reversed, the image appearing quite differently—for reliefs are now expressed as hollows—in a somewhat remarkable manner, called "pseudoscopic vision," the subject being again referred to in footnote 3, on page 259.

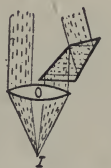


Fig 242. Wenham's Prism.

Owing to the great interest taken in later years in this stereoscopic method of viewing objects, much attention has been given to the subject by opticians in general, a great impulse being made by the exhibition of an excellent arrangement devised by a continental manufacturer some years before the war, which could be employed for low and high powers.

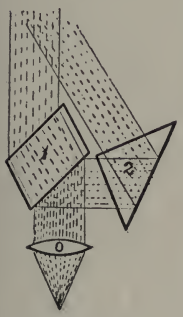


Fig. 243. Powell & Lealand's Prism.

To recapitulate somewhat, and yet to amplify what has already been said (which is necessary so that what follows shall be thoroughly understood), we must point out that hitherto binocular microscopes may be arranged into three groups, each having their instrumental and practical differences.[1]

Type 1 is that already described, and which was invented by the illustrious Wenham. In Fig. 242 is repeated, although in a different form, what is shown in Fig. 149, page 255. It is here seen that the full

[1] The substance of what here immediately follows (including the explanatory figures) is taken, by the kind permission of the writer, Mr. Conrad Beck, from his interesting brochure upon the subject, which the student is invited to read in its entirety.

beam of light emerging from the object glass is bisected, one half going to one eye, and the other half to the other eye, this being thought a necessity at that time. As a matter of fact, vision in this arrangement can never be equal to that of the monocular, because reducing the size of the beam of light which forms the image, necessarily reduces the resolving power of the objective. The arrangement is also at fault, because high powers can never be employed, as the prism cannot be placed near enough to the back lens to properly bisect the rays. Further, the instrument cannot be constructed for the short tube-length. Type 2 is an improvement, because the beam issuing from the lens is not bisected, and the arrangement was so constructed that some light from each portion of the object-glass was utilised by each eye. The prism, invented we believe by Powell & Lealand, is shown in Fig. 243. Here it is seen that the whole of the rays from the back lens impinge on a glass plate (1, Fig. 243), and the greater part pass through the thick glass emerging in a direction parallel, and almost continuous with the original direction; but a certain quantity is reflected into the second tube of the instrument, in the manner shown—see the prism 2 in the same figure. It will be quickly noticed in this type the resolution is equal to that of the monocular instrument, because the size of the beam which forms the image is not reduced. Putting aside that the tube must be long—which is considered by many a great drawback—the main defect is that the light reflected is so ex-

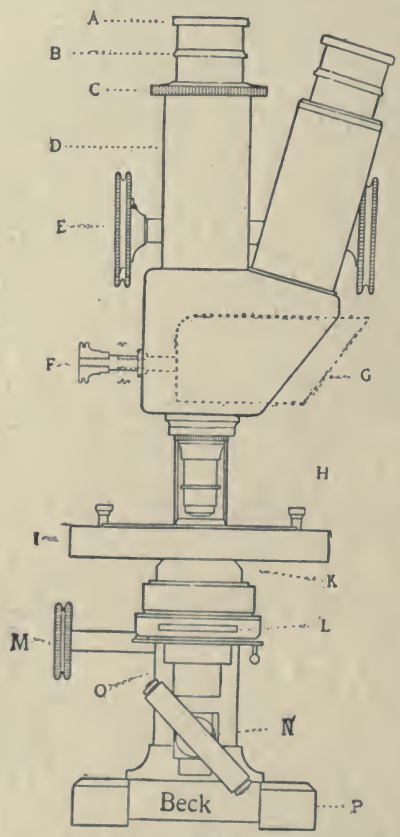


Fig. 244A.—Beck's Binocular.

A. Eyepiece.
B. Draw-tube.
C. Revolving flange for raising draw-tubes.
D. Body.
E. Course focussing adjustment.
F. Prism box knob.
G. Sliding prism box.
H. Object glass.
I. Stage.
K. Substage condenser.
L. Iris diaphragm.
M. Substage focussing adjustment.
N. Mirror.
O. Pillar.
P. Base.

ceedingly feeble, there being a difference of about six to one between the eyes.

Type 3 is that brought about by Zeiss and called the Greenough Binocular, after its inventor. It is illustrated and explained on page

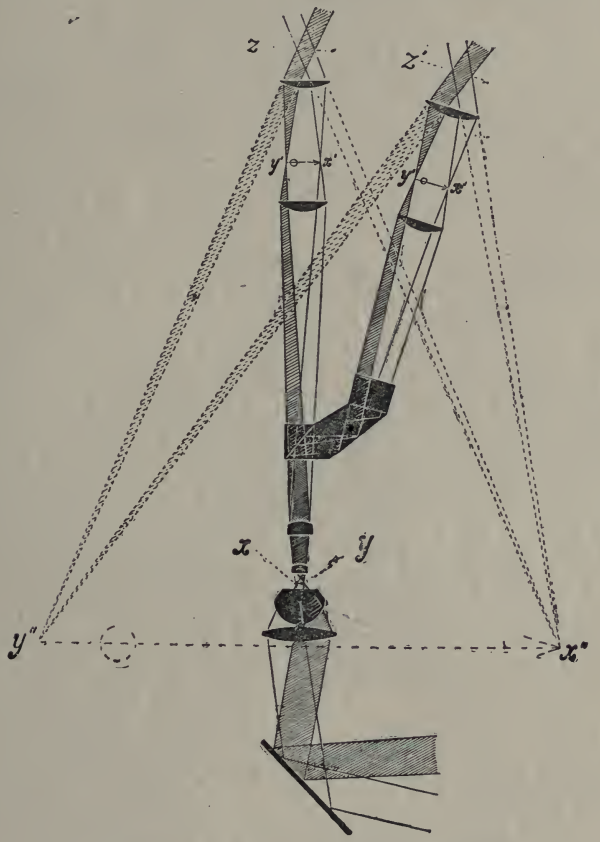


Fig. 244B.—Path of Rays in Beck's Binocular.

$x\,y$. Object.
$x'\,y'$. Image formed by object glass.
$x''\,y''$. Virtual image formed by eyepiece.
$z\,z'$. Ramsden discs—conjugate images of back equivalent plane of object glass.

257. The only objection to this form is that, whilst perfect for low powers, provided they are carefully matched, it cannot be employed with those of short focal length in any form or shape.

In the Beck Binocular, shown in Fig. 244A and 244B, we see an arrangement of quite a novel construction. Above the object-glass is a

prism shaped as shown in Fig. 245. The whole of the light passes through the surface of the glass B A to a surface E A, which is coated with a semi-transparent layer of silver. Part of the light passes *through* this to one tube, whilst the other part is *reflected* into the second, as shown in the dotted lines. Thus the full-size beam goes to form each image, and hence no lack of resolution occurs from its bisection, and each eye has, too, in consequence presented to it the maximum of resolution. The direct effect is that each image is equally bright, for this is carefully attended to in the process of manufacture according to the amount of silver deposited. The additional length of the path of one ray over the other is regulated by the introduction of a piece of plate glass which is placed at E F, and increases the focal length of the beam as explained in Fig. 246, where the ray, ordinarily focussing at A, is shown prolonged by this means to B. If this piece of glass be made of exactly the required thickness, equal focus and equality of magnification are obtained in each eyepiece.

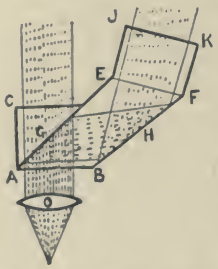


Fig. 245.—Beck's Prism.

The interocular distance is varied by turning the milled ring C in Fig. 244A; it causes both tubes to move in or out, adjustments being capable from $2\frac{1}{4}$ to $2\frac{3}{4}$ inches. The standard tube-length is 160 mm.

The stereoscopic and pseudoscopic effects are brought about by simply looking into the instrument closely, or by removing the head a very short distance away, no limiting diaphragms being required. The peculiar ease of obtaining this change of "effect" is brought about by one tube being so much inclined, whilst the other is quite straight. Pushing the prisms away by using the knob F (see Fig. 244A), the instrument becomes a first-class monocular of short tube-length.

Fig. 246.
Glass increasing Length of Rays.

The path of the rays is shown in Fig. 244B.

The Watson-Conrady Binocular Attachment

In this new binocular attachment a novel departure has been made. The arrangement of the optical system appears to be similar to that employed by Abbe for dividing the light coming from the objective (see page 258), the lateral prism being provided with a glass extension. Beyond this the following ingenious method is adopted to allow of adjustment for width of eyes without any alteration of tube-length, the plan usually adopted in most instruments of the older type.

The lateral prism and the tube carrying the lateral eyepiece are mounted together on hinged bearings set in such a position that *the beam of doubly reflected light always occupies the same position with reference to the axis of the lateral tube.* By turning the screw C in Fig. 247, the binocular tube is gently altered in its angular position until the user's width of eyes is suited. If, then, it be necessary to change the tube-length, the interocular distance can be at once easily reset.

The arrangement cannot be used on all patterns of microscopes on account of the size of the prism.

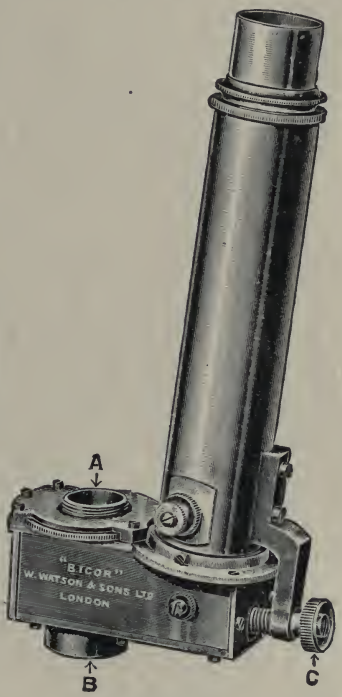


Fig. 247.
"Bicor" Binocular Attachment.
A. Screw to attach to monocular body.
B. Screw to receive objectives.
C. Screw to adjust for width of eyes.

On the use of Short Wave-length Screens with Powerful Illuminants

To be able to use a blue screen has long been the desire of the microscopist (see page 169). The advantage gained in resolution is very great, as already explained. Experimentally this is illustrated by two reproductions in Plate XIX., where Fig. 3 shows Amphipleura pellucida and another diatom photographed with red light, and Fig. 4 where they are seen as they appear with blue illumination. The lines in Amphipleura, which in this specimen are about $\frac{1}{110000}$ of an inch apart, are not represented at all in the first figure, but are distinctly visible in the second.

Unfortunately, however, immediately the student attempts to make the experiment, he finds himself confronted by a great physiological difficulty not easy to overcome. This consists in the fact that the human eye does not appreciate every colour alike, the retina not being equally sensitive to all the different wave-lengths of the spectrum. A simple diagram, Fig. 248, shows this very plainly. Here it is readily observed that the *greatest* appreciation lies approximately in the yellow-green (often called the apple-green), which, it might be mentioned in passing, is the reason this colour is selected for the correction of all visual objectives, as previously explained. Following the course of the

curve, it will be further seen that, as the colour approaches blue, the "seeing" power of the eye experiences a great drop, so much so that about G in the blue it becomes exceedingly limited, and all appears to us as black and dark, especially as we get farther and farther into the violet at H. About 470$\mu\mu$ wave-length (4700 in tenth-metres) is the usual limit, so that no blue screen of shorter wave-length is of any service visually, because we cannot see the image in it.

It has been our desire to find a blue screen (even if passing some green) that could be employed with the ordinary oil lamp used by many microscopists who cannot obtain the electric light or even gas. We are unable to do this at present, at least not one that will suitably show the dots in Amphipleura, but we are able to suggest two blue films that are suitable for use with powerful illuminants.

The first, the more transparent of the two, is called number 48 film in the list of those sold by Kodak, Ltd. It transmits, when used alone, some of the red end of the spectrum (besides some green which does not materially interfere with its action), and to remove this —for it spoils the visual image as seen in the ocular —has to be combined with another film, number 38. Sometimes two of this number are required, and even a third at times. The 38 screen scarcely affects the appearance of any part of the spectrum save the red end, which it suppresses. It must not be thought, however, that this compound screen formed by the joint action of numbers 48 and 38 produces a pure monochromatic blue or blue-violet, because it passes a great deal of green, but as the *maximum transmission* lies at about 4700 wave-length (Ang. units), so it appears to be the best procurable at the moment, for no really pure blue (so far as we know) is obtainable, except by employing a prismatic arrangement such as that described on page 170.

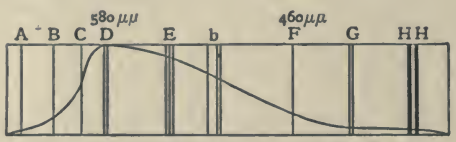


Fig. 248.—The Sensitiveness of the Eye to Different Colours.

A to C. Deep red to pale red.
C ,, D. Pale red orange to yellow.
D ,, E. Yellow apple-green to green.
E ,, G. Green to deep green.
G ,, F. Deep green to blue.
F ,, HH. Blue to deep violet.

A second blue film called "L" is much denser, but it passes less green, and its maximum transmission lies at about 4600 wave-length.

It is obvious, from a study of Fig. 248, to utilise these blue screens it is necessary to employ very powerful illuminants, such as the Argus arc lamp, or an ordinary mixed oxy-hydrogen gas jet.

Taking the Argus lamp (about 700 candle-power) first, Fig. 108, page 167, it is used in the following manner:—Having placed the lamp so that the illuminated area of the transverse or positive carbon (called

"the crater") is about 20 to 22 inches from the mirror of the microscope, and the condenser of the little lamp (having usually a focal length of about three inches) at about four or four and a half inches from the crater, the appearance presented on looking down the tube of the instrument—the ocular being removed—is examined. Before actually doing this, it is necessary to interpose both blue screens, or one blue and a neutral tint, film number 98, between the draw-tube and the eye, or between the condenser of the *lamp* and the mirror, as the light is too powerful for the naked eye.

If now the back lens be equably filled with light, the observer can proceed to employ the ordinary method of obtaining oblique illumination, but if, on the contrary, the light be apparently collected into a kind of star, occupying the central and part of the intermediate zones only, then difficulties arise.[1] The cause of the trouble may be two-fold. First the lamp, with its condenser, may be too near or too far from the mirror (usually too near). Let it be moved in both directions and the result examined. Presuming neither position has an equalising and distributing effect, the trouble arises from the condenser not being constructed to work with such a thick slip. In our case we have three tops to the Holos condenser, which we constantly use when necessity demands, for occasionally a fine specimen is found on some old slip, and these were mostly made of stout glass "to prevent their easy fracture!" Fortunately there is nearly always another way of getting over the difficulty which saves purchasing a condenser of different construction, or another top to the one already in use.

When blue light is required to assist in obtaining the highest and finest resolution possible, it necessarily demands the employment of oblique illumination. This means that the light issuing through the back lens of the objective shall be limited by a suitably shaped diaphragm to one edge of it, the position chosen being that which will furnish simultaneously two diffraction images in adjacent parts of the field similar to that shown in Fig. 125, page 198. In this instance, however, instead of doing this, the small star of which we have spoken is—by moving the mirror—placed there instead, no diaphragm of any kind or of any shape being used at all! We have seen excellent photographs of Amphipleura taken in this way which showed the dots very perfectly. It is well to bear in mind when obtaining oblique illu-

[1] It should be noted that when a very strong light is used, and only a pale screen employed, the back lens may often appear to be fully illuminated, and equally too all over its entire extent, but this arises from the great glare. It can be easily recognised as incorrect when present, if two deep screens be employed, when the star-like appearance already spoken of will be readily recognised. Care should be exercised that this mistake is not made, for it is easy to fall into the error.

mination by this method, which with practice also yields excellent visual results, that the perfection of the final image is to be sought by revolving the specimen about the axis of the instrument, or by very slightly altering the position of the mirror, the substage diaphragm being left undisturbed. We think this unconventional means of obtaining oblique light yields a brighter image than that secured by the ordinary method.

The limelight requires no special mention save that it must be in its most powerful form, namely, the mixed jet, and that the limes shall be of the very best quality. A condenser of the same type as that used with the little arc lamp will also be required, for no lamp of which we are aware is distinctly arranged for microscopic work. The same screens can usually be employed.

The Pointolite lamp of 100 candle-power is not powerful enough to be employed with the 48 and 38 films, and the best to use is a cemented form sold by the Kodak firm called number 38aM, with which we have seen, though somewhat imperfectly, the dots in Amphipleura. The 500 candle-power lamp of the same make is certainly a competitor with the Argus arc lamp just described, but it requires (as well as the 100) the addition of a condenser similar to that fitted to the Argus arrangement. The 500 candle-power lamp is not so brilliant as the arc, being many candles less in illuminating power, hence it can only just be used with the 48 and 38 films.

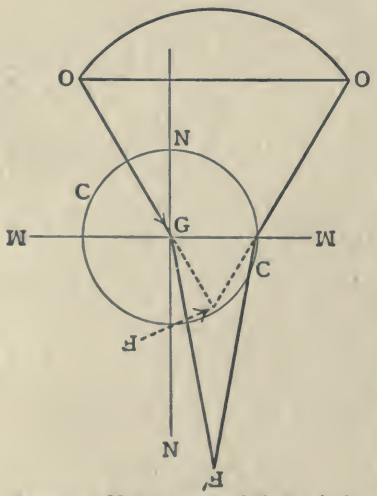


Fig. 249.—How Depth of Focus is increased by the use of Denser Media in Mounting Specimens.

How Depth of Focus is increased by the use of Denser Media in the Mounting of Specimens

On page 99, Fig. 83B, is shown how the cone of rays meeting at F is produced to F'. It has been found difficult by some readers to quite understand how such change is effected. Let the diagram be turned upside down, as shown in Fig. 249, and let the point of the meeting of the ray O with M be called G. From this as a centre let the circle CC be described, and the normal NN be drawn at right angles to MM. Seeing that a ray passing from a rare into a denser

medium is always turned towards the normal, so the ray OG, instead of passing straight along as shown by the dotted line to F, is deflected to F′. The same thing happens on the other side of the cone, producing the elevation to F′ as shown in Fig. 83B.

The Index of Visibility

Besides the increase in depth of focus, it has been pointed out before that, in the mounting of diatoms, the greater the difference between the refractive index of the mounting medium and that of the silicious frustule, the better the resolution, and consequently the greater facility afforded for the discovery of details. It may be said, in fact, that *any* object inserted in a medium is visible according to the amount of *difference* between the refractive indices of the medium and of that object.

The silica of diatoms has a refractive index of 1·43, whilst that of water is 1·33. The difference between these two is 10, which we believe was first christened by the late Dr. Van Heurck as the "Index of Visibility." Canada balsam has an index of refraction of 1·53, hence its index of visibility is 10. Pure styrax, with its index of 1·58, reaches a higher index of visibility amounting to 15. Mono-bromide of naphalene, the refractive index of which is 1·66, has a visibility of 23, but iodide of methyl, with its refractive index of 1·74, the still higher level of 31. If this oily substance (which should be quite colourless) be saturated with sulphur, its index of refraction is still further raised to 1·78, and so the visibility figure is increased to 35. But all these "visibility indices" are put in the shade when that of artificial realgar is being dealt with, for its refractive index reaching to 2·55, the highest visibility index known for any fluid medium is arrived at, namely, that of 1·12.

Annular Light

Annular light, which consists in occluding the centre of the cone of rays coming from the condenser, either by the use of a Traviss's expanding stop (page 174), or by a suitably sized diaphragm made in wheel form as for darkground illumination, was formerly often spoken of as being useful under certain circumstances in place of oblique light. It has fallen out of use, as most microscopists have found the same as we have ourselves been led to believe, viz., that the results so obtained are never so fine as represented. Mr., now Prof. Conrady, has given his attention to the matter, and explains the reason why such deficiency in effects is reasonably to be expected. We give his explanation in his own words. "The diffraction spectra produced by

regular structures form a perfectly fixed pattern when seen at the back of the objective, that is to say, however we may place the direct ray (by use of the oblique-light arrangement), the diffraction-spectra travel an exactly similar amount, and rigorously keep their distances from the direct light and from each other. From this it follows that to annular direct light there corresponds a set of annular diffraction spectra, of which, however, only a certain part can be seen at the back of the objective, if, as usual, the annular light fills the marginal zone of the objective. In Fig. 250, under these conditions, *only the portion of the diffracted rings falling within the direct ray* would be visible.

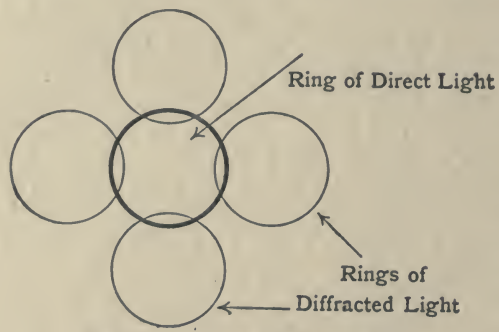


Fig. 250.—Annular Light explained.

"Further, only similarly placed portions (or portions which are in the same *azimuth* of the several rings) of the various rings can interfere and produce an image, and other parts admitted are useless for purposes of resolution.

"Dot-resolution is only possible if similarly placed portions of direct light and of two diffracted rings are simultaneously admitted by the objective." From these remarks it is pretty evident that annular light can never be expected to be so effective as oblique illumination.

Rheinberg's Screens

Owing to the "Illustrated Annual of Microscopy for 1899" being now so difficult to obtain, and as we have been asked for details of Mr.

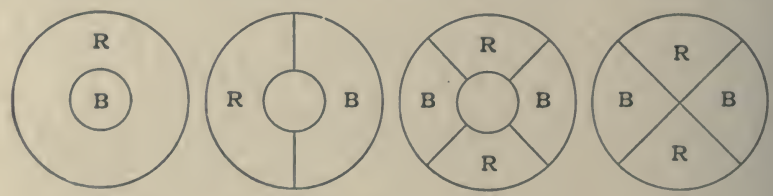


Fig. 251.—Rheinberg's Screens.

Rheinberg's discs—for their use seems growing in popularity—we are showing through his courtesy, in diagrams 1, 2, 3, 4, in Fig. 251, the

leading forms, although any design of figure furnishes interesting results. No. 4 requires mounting on a glass disc. This and No. 3 are useful to show the horizontal and vertical strands of a silk handkerchief illuminated with different colours. Certain changes, too, of the mirror will effect peculiar alterations of the colours, particularly with No. 4. The letters R and B denote red and blue, but other colours can be used, green and red suiting some specimens extremely well.

Using a Nicol's Prism over the Ocular to improve the Definition

For some little time it has been stated by different microscopists that on certain occasions they have noticed an improvement in the definition of diatoms when a Nicol's prism was placed over the ocular. Whether we could corroborate this, and to what extent, and under what circumstances it occurred, became the subject of inquiry, and the object of making several experiments. We were not aware at the time of a statement in "Carpenter" (page 381 of the eighth edition, 1901), where it laconically remarks as "a curious and hitherto unexplained fact, that when resolving striæ or lines with oblique light, the effect is much strengthened by placing a Nicol's prism over the eyepiece." Hence the subject cannot be regarded in the nature of a discovery, although, up to the present, nothing so far as we know in the nature of an explanation has been offered.

Quite soon after the commencement of the experiments it came somewhat in the nature of a surprise to find that any betterment in definition was limited to the sharpening up of closely approximated *lines* in a specimen, and that no increase in the distinctness of vision was noticeable when *dots* were under examination.[1]

Further, it soon became evident, which is not easy to understand, that even this limited improvement was not a constant quantity; in fact, it was not common with all types of specimens, for in some instances, such as with the lines in Amphipleura pellucida, nothing of the sort appeared to take place, while in other instances, such as with the striæ in Nitzschia singalensis, a distinct and pronounced sharpening up was plainly evident. Moreover, it was quickly recognised that in any case of improvement, to witness the maximum effect, the nicol had to be revolved upon its axis to find the "best position"; but this, of course, merely verified the fact that the rays being dealt with were polarised beams.

Further, the experiments seemed distinctly to show that the most

[1] This peculiarity has been previously noticed, we find, by other observers, notably by Dr. Burton-Brown, who has paid some little attention to the subject.

marked effects occurred when using highly apertured objectives like an immersion twelfth; that but little if any improvement was noticeable with a sixth, and that with quite low powers, such as an inch, the reverse seemed to obtain, for there was a falling off both in definition as well as in brilliancy of the image. Improvement then, resulting from the use of this prism, seemed limited to certain types of valves, and for the most part was restricted to the use of very highly apertured combinations.

With respect to the theoretical consideration of the case, a paper in the *Journal of the Royal Microscopical Society* for the year 1905, page 552, by Prof. Conrady, states that "diffracted light at a considerable angle from the direct light is polarised," it being further pointed out as a remarkable confirmation of the diffraction theory. It appears, too, that the subject does not lend itself to exact theoretical investigation, as the complete explanation of polarised diffracted light calls for mathematics of a high order, and that even then it cannot be applied to concrete problems, as an exact knowledge of the minute structure would be required, of which—especially in the case of diatoms—nothing is *really* known, or even knowable for *certain*. But it seems, however, of interest to show roughly how the effect in question arises, and this may be best done by sketching the ether-vibrations, and remembering that only those which are transverse produce a light effect. Suppose SS in Fig. 252 to be a slit, and the vibrations in the plane of the paper as shown by the arrow. If now the diffracted light went off in the direction of the arrows, there would be only longitudinal vibrations which do not produce a light effect. Light then, polarised in the plane of the paper, would produce no diffracted light in the direction shown, but light polarised at right angles to the plane of the paper would. The result is that when diffraction takes place at great angles, the diffracted beams are weakened compared to the direct. By applying an analyser in the direction which transmits the diffracted light, then only the direct light—which is unpolarised—will be weakened, and so contrast will be improved.

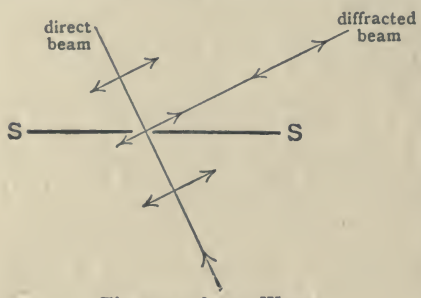


Fig. 252.—Ether-Waves.

Based on this simple theory—which has been suggested by Prof. Conrady—the effect should be at its maximum when the angle between direct and diffracted beams is 90°. With balsam and monobromide

mounts this would be the case—in other words, with test-objects suitable for a twelfth—and that is confirmed by our experiments. With low-power objectives, the angle between the direct and the first diffracted beam is, however, too small to produce a sensible effect, which again is in accordance with the results experimentally ascertained.

The form of nicol which gives the best results is one that is square ended, reminding one of a Prazmowski's prism, having about a 6 mm. aperture, with the top and bottom faces at right angles to the microscopical axis, and short, for if the spar be too long, the field is unpleasantly curtailed, and often limited to little more than a broad slit. It has been suggested to use a polariser instead of an analyser, and we have seen it recommended. We cannot endorse this suggestion, for if the prism be mounted in the iris fitting, as usual in all or nearly all instruments of foreign manufacture, moving the spar round to obtain "the best position" alters the position of the diaphragm which is being used to obtain the oblique illumination, and all the arrangements have to be changed. In some English stands this objection does not obtain, as the prism is in a sleeve of its own, which can be separately revolved apart from the iris diaphragm. Even then we think the plan objectionable, as the light which passes into the object is charged with any imperfections resulting from an unsymmetrically cut prism, which, when it is revolved, it is easy to understand may seriously affect the definition of a highly apertured objective. In the case of an analyser placed *over* the eyepiece, this is not productive of similar trouble, or certainly not to the same extent. The prism can be placed directly over the objective, and is indeed so recommended we believe by Commander Ainslie, who has paid some attention to this subject; but of course it implies a special arrangement, and it is better to have the nicol especially cut for use in this position. For convenience, simplicity, and rapidity of application, we prefer placing the prism over the ocular. Wherever placed, what restricts the improvement in definition to certain diatoms, or why increase of clearness of vision is limited to the "lines" and does not extend to the "dots," is not immediately apparent.

Summary of the Colour Corrections required in Different Objectives

With respect to the colour corrections required for objectives to merit the distinguishing titles of Achromatic, Semi-apochromatic, and Apochromatic, it may be of interest to enumerate those that obtain in the following manner:

In an ordinary flint and crown objective, two colours are united, but for one zone only.

In the semi-apochromatic, two colours for two zones.
In the complete apochromatic, three colours for two zones.

In the ordinary flint and crown objective, the two colours usually united are at C and F in the spectrum, whilst the zone selected lies at about two-thirds of full aperture.

In the semi-apochromatic the two colours united are also at C and F, the zones united being the centre and the outer.

In the apochromatic the three colours vary somewhat, being dependent on the glass used and the fluorite, whilst the zones of unition are the same as the preceding, viz., the central and the outer.

In all the above cases, especially the semi-apochromatic, the individuality of the artist in selecting his wave-lengths to be united is always in evidence, and can, with experience, be easily recognised when using the Abbe test-plate, see pages 71, 367-8.

Concerning the Magnification by Different Colours in Microscopical Objectives

In ordinary achromatic lenses the blue end of the spectrum furnishes a larger image of the object than the red because the rays are of shorter focal length (just the same as in a hand magnifier, an inch magnifies more than one of two-inch focal length).

In this type of combination the absolute difference of the magnification may vary almost to any extent, but in an apochromatic it is not greater between C and F in the spectrum (that is, between wave-lengths 6563 and 4862 approximately) than about 1·2 per cent., or, say, one part in eighty-three.

Converting Wave-lengths in Tenth-metres into Waves per Inch and Vice Versâ

If the number 254,000,000 be divided by the term in tenth-metres, the result is the "number of waves to the inch." *Vice versâ*, the figures in "waves to the inch" divided into the same nine-figured quantity furnishes the result in "tenth-metres."

The figures 254,000,000 are obtained in the following manner:—

1 inch = 25·4 millimetres,
1 micron (μ) = $\frac{1}{1000}$ part of a millimetre;
hence there are (25·4 × 1,000) 25,400 microns in an inch.

But there are 1,000 double *mūs* ($\mu\mu$) in a micron (μ), therefore there are 25,400 × 1,000 = 25,400,000 $\mu\mu$ (double *mūs*) in an inch. The additional cypher to make 254,000,000 is required, because the tenth-metre is one more place down the scale, see pages 156, 268, 269.

Rheinberg's Eyepiece Micrometers, Stage Micrometers, Crosslines, etc., for the Microscope

Graticules are discs of glass, with measuring scales upon them of various kinds, which, besides being used on the microscope as eyepiece and stage micrometers, are employed as an essential part or adjunct of nearly every optical instrument, more especially those used in war.

The manufacture of these graticules makes the highest demand upon extreme accuracy and precision in many ways, for it is obvious if reliance is to be placed upon them to such a degree, and they are found faulty, their presence is worse than useless. Many measures too are microscopic in themselves, hence the actual divisions have to be true, really, to within one or two tenths of one per cent., whilst the thickness of the actual lines—which is predetermined—may be required to be one-thousandth of an inch, a two-thousandth, or, even in some instances, a five-thousandth of an inch in magnitude.

Until the advent of the war, most graticules were made in Germany, hence, when hostilities had started, it became evident something must be done immediately in England to compete with the situation. With this object in view, it redounds to the credit of Mr. Rheinberg—whose name is so well known in scientific circles—for commencing at once, assisted by his brother, a research as to how graticules could be made by photography, which meant quickness of production, in contradistinction to the slow, old-fashioned method of ruling each line and then etching it on to the glass. After fifteen months' research, he and his brother succeeded in producing a grainless photography, which was in itself a great achievement; but it was followed by a still greater discovery—viz., that of producing the divisions by what is now known as filmless photography. The advance is enormous, as the photographic image is produced in untarnishable metal *actually in the surface layer of the glass itself*, and hence is absolutely permanent and irremovable by any ordinary means whatever.

At the moment of writing, most scales required by the microscopist are in existence, but others of all kinds are in progress of manufacture, both in millimetres and inches. They are obtainable at any opticians. Messrs. Angus & Co. usually keep a large stock.

New Eyepieces, by Messrs. Swift & Son

Some new oculars are made by Messrs. Swift & Son of a very remarkable character. They possess several desirable features which we do not think have been combined

Fig. 253. "The Medial Ocular."

in one system before. One is, they exhibit such a pronouncedly flat field, particularly noticeable when examining an object from the edge to the centre, for there seems no necessity to adjust the focus. They have also a very conveniently distant eye-point, so that the eye is at quite a long distance from the front lens. We have not been able to examine the whole series, but, judging from the one that we have experimented upon, it really appears to be usable for all powers up to a sixth with perfect success, and we have the greatest difficulty to discover that it does not suit an apochromatic as well as an ordinary semi-apochromatic; the low-power combinations certainly do. So, too, with an immersion twelfth, the image is exceedingly fine, an Abbe test-plate being employed. They are called "the mediar oculars," and one is shown in Fig. 253.

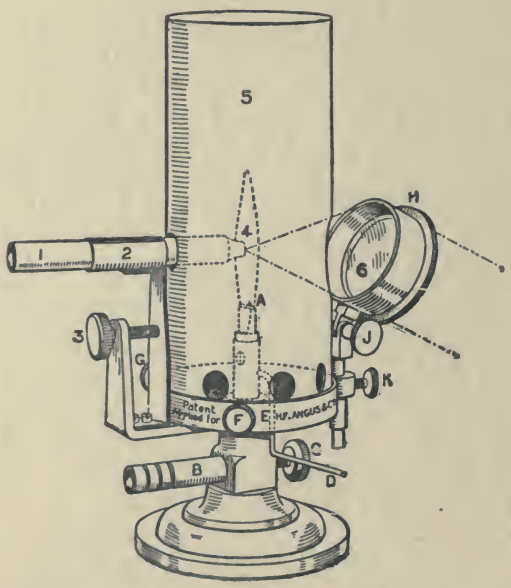


Fig. 254.—The Barnard Incandescent Gas Lamp.

1. Metal tube, containing incandescent material rolled in the form of a cigarette, protruding about ¼ inch when in use; a lead pencil will be found a convenient plug with which to push the material forward in the tube. When first used, the tube should be rotated in the cradle, 2, until the incandescent material presents a convex surface to the flame.
2. Cradle holding the tube, which slides easily in it, this sliding movement serving to approximate the incandescent material to the flame.
3. Fine adjustment screw, by means of which the relative position of incandescent material to flame can be adjusted exactly.
4. Bunsen flame emitted from the slit, A, it is fed through the gas connection, B, and controlled by the milled head, C, which regulates the supply of gas, and the lever, D, which regulates the supply of air.
5. Metal chimney enclosing the flame supported by the gallery, E, to which it is clamped by the screws, F, G.
6. Bull's-eye condenser adjustable for focus by sliding in sleeve, H, and for inclination by hinged joint with clamping screw, J; it is attached to the gallery of the lamp by clamping screw, K.

NOTE.—In the latest pattern the jet is a compound one and more powerful. It is manufactured by Messrs. H. F. Angus & Co.

Barnard's Incandescent Lamp of High Power

Mr. Barnard, President of the Royal Microscopical Society, has recently arranged a highly ingenious and powerful gas lamp which appears to be all that is desired. A full account will be found in the *Journal of the Royal Microscopical Society*, Part IV., 1918. It is

not, however, sufficiently powerful to be used with the blue screens and high magnifications.

A New Gas Lamp for the Microscope

Mr. Traviss has invented an ingenious lamp with a very small illuminated area for the microscope, which seems, so far, to be giving much satisfaction, although at the moment of going to press it is still somewhat in the experimental stage so far as relates to its final form. It consists of a disc of incandescent material that is attached to a wire (capable of being adjusted), upon which plays one small jet (or more) of ordinary house gas. If the pressure be as good as it should be, this little disc becomes highly luminous, and gives a very steady and uniform light, and the disc lasts a very long time without cracking. It is not a high-power illuminant, and is called the Thorium disc lamp.

Some New Objectives

During the war little has been done in introducing new objectives, and those which were computed and made either just before hostilities commenced, or whilst they lasted, are for the most part unobtainable. As we were going to press, however, a few were brought before our notice, most of which have been made by Messrs. Swift & Son. Their apochromatic series, several of which we have seen and tested, justly merit the attention of all microscopists, and we regret being unable to illustrate their performance in this issue. The 1·30 homogeneous immersion is an exceedingly fine lens yielding an irreproachable image of Amphipleura pellucida in dots, and has a very fine colour correction after the style of Zeiss, Leitz (their latest pattern), and Koristka, rather than that of Hartnack and Reichert. The lower powers also deserve very great praise. The same firm have also introduced some low-power (25 and 16 mm.) water immersions for the examination of aquatic organisms which appear to give great satisfaction, supplying a want that has been long felt. Messrs. Watson & Sons, before the commencement of the war, began to make their apochromatic systems, and we had then the privilege of examining a sixth of very great excellence. We put it through several tests, and compared the image with that afforded by combinations by Zeiss, Leitz, and Reichert, and are pleased to say it was impossible to distinguish its performance from any of them. We offer the firm our hearty congratulations, and are glad to hear that a completion of their series will be forthcoming as soon as circumstances permit. Their new Versalic $\frac{1}{12}$th is mentioned elsewhere in this book. It has been designed for hard laboratory use,

and is said to be provided with a new method of fixing the front lens that prevents almost entirely the possibility of its being pushed in during rough treatment.

Charles Baker has brought out a few new combinations, and we have just examined a very remarkable $\frac{2}{3}$rds of irreproachable performance. So fine is this, that with green illumination it is very difficult, if indeed possible, to distinguish the image from one furnished by any apochromatic of the same focal length.

The old firm of Wray, which closed down its series of microscopic lenses during the war, is now once more before the public, but at present limits the output to photographic combinations. Their "Lustrar" is a very remarkable combination, giving an exceptionally flat field with perfect definition at full aperture, F/6·3. In short focal lengths it would be very suitable for photographing entire insects, etc., such as we have already spoken of.

Abbe's Original Formula for Immersion Oil

29 grams of *white oily* Tacamaque (Guibert), sometimes called Tacamahaca (*Fagara octandra*), dissolved in 22 c.c. of cedar oil (*Juniperus Virginiana*), and diluted with 14 c.c. of castor oil (*Ricinus Communis*).

James Swift & Son's "Improved Climax" Fine Adjustment

This was the first micrometer movement in which the controlling milled heads were placed horizontally, parallel to those of the coarse adjustment, and it has proved itself most satisfactory. The action of the mechanism is apparent from the illustration. The micrometer screw A is fitted horizontally, and carries a bronze lug B; this lug is prevented from rotating, as a groove cut along it bears round the steel bar C. Hence on the milled heads H, H^1, being turned, the lug travels either to the right or left. One end of the lever D bears on a flat surface cut on the lug, so that the movement is conveyed, at a reduced speed, to the roller E, which is attached to the body-slide. The pitch of the micrometer screw is ·5 mm., and the lever ratio is 5 to 1, therefore one complete revolution of the milled heads raises or lowers the body ·1 mm. The drum is divided into 100 parts, so turning the milled heads through one division of the drum corresponds to a movement of the body of ·001 mm. or 1 μ. This adjustment has the comparatively long range of 3 mm., and all parts are so carefully made and calibrated that it is possible by its means to measure refractive indices accurately to the second place, and approximately to the third place of

decimals. As the number of its working parts is reduced to a minimum, and all are completely enclosed, it is practically impossible for this adjustment to become deranged. Owing to the design explained above, it can show no sign of backlash or loss of time on suddenly reversing its action while focussing, even after years of constant use; this backlash, we may say, is generally an inherent trouble with similarly placed adjustments which have an Archimedean worm and wheel in place of the aforementioned micrometer screw. As with the coarse adjustment, turning the milled heads from the observer always lowers the body; turning them in the opposite direction always raises it. The adjustment automatically ceases to act should the objective touch the cover glass, so there is practically no chance of injuring the specimen or damaging the lens.

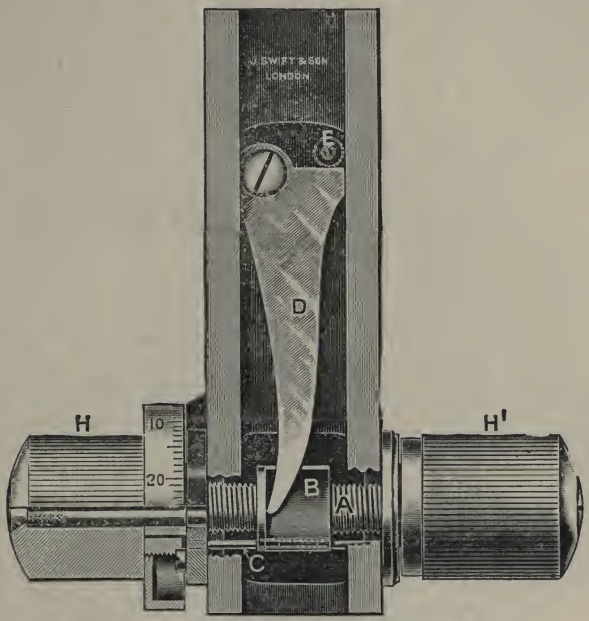


Fig. 255.—"Improved Climax" Fine Adjustment.

Details concerning the Power and Focal Length of Compensating and Ordinary Huyghenian Eyepieces

The approximate focal length of compensating eyepieces for the long body microscope.

Power . . .	× 2	× 4	× 8	× 12	× 18	× 27
Focal length in mm.	135	67·5	33·7	22·5	15	10
Inches . . .	5·3	2·6	1·33	·89	·59	·39

For the short body.

Power . . .	× 2	× 4	× 6	× 8	× 12	× 18
Focal length in mm.	90	45	30	22·5	15	10
Inches . . .	3·54	1·77	1·18	·89	·59	·39

The focal length of Huyghenian eyepieces.

Power . . .	× 1	× 2	× 3	× 4	× 5
Focal length in mm.	50	40	30	25	20
Inches . . .	$1\frac{31}{32}$	$1\frac{9}{16}$	$1\frac{3}{16}$	1	$\frac{25}{32}$
Magnification .	3	4	5·5	7	9

Index of Refraction of Different Substances

Substance.	Index of Refraction.
Water	1·334
Fluorspar	1·433
Diatom silex	1·434
Petroleum	1·457
Oil of turpentine	1·474
Naphtha	1·475
Oil of olives	1·476
Oil of linseed	1·485
Castor oil	1·490
Chloride of tin	1·503
Dammar	1·520
Canada balsam	1·526
Oil of cloves	1·533
Oil of fennel	1·544
Rock crystal	1·545
Nitro-benzene	1·558
Oil of cassia	1·578
Styrax	1·582
Quinidine	1·602
Balsam of tolu	1·618
Oil of cinnamon	1·619
Bi-sulphide of carbon	1·630
Iceland spar	1·657
Piperine and balsam	1·657

Substance.	Index of Refraction.
Mono-br. of Naphthalene	1·658
Piperine	1·681
Sulphur in methylene di-iodide	1·778
Phosphorus	2·224
Diamond	2·470
Realgar (artificial)	2·549

A few of the different types of glass now manufactured by Messrs. Chance Brothers[1]

Boro-silicate crown	1·5087
Zinc crown	1·5149
Soft crown	1·5152
Telescope crown[2]	1·5153
Hard crown	1·5155
Extra light flint	1·5290
Light barium crown	1·5407
Light barium flint	1·5515
Light flint	1·5632
Medium barium crown	1·5744
Dense barium crown	1·6118
Dense flint (No. 361)	1·6214
Very dense flint	1·6469

Wave-lengths of the Different Parts of the Spectrum, with the Maximum Transmission of Different Screens in Tenth-metres, and the Corresponding Approximate Number of Waves to the Inch of the same

Tenth-metres.	Waves to the Inch.
Red, 7606	33,394
Red (B), 6870	36,972
Orange (C), 6563	38,702
Yellow (D), 5893	43,102
Apple-green, 5500	46,182
Blue (F), 4861	52,253
Violet (H and K), 3950	64,304

[1] For further details the reader is referred to the long list published by the firm.

[2] This glass has been made to match the constants, calculated by Prof. Conrady, of the Imperial College of Science and Technology, and has the peculiar advantage that when combined with dense flint type (No. 361), small cemented objectives (with crown leading), corrected for spherical and chromatic aberration, are obtainable by the use of three curves of equal radii and a flat surface.

These figures are sufficiently accurate for the working microscopist. White light contains the whole range of the spectrum; but microscopical experience gives its monochromatic equivalent as about 5347 tenth-metres, equivalent to 47,500 waves to the inch.

With reference to the films used in conjunction with the small arc light, No. 61, green, has a maximum transmission at about 5450 tenth-metres, equivalent to 46,605 waves to the inch; No. 48, blue (often called C Filter), has a maximum transmission at about 4700 tenth-metres, equivalent to 54,042 waves to the inch; No. L at about 4600 tenth-metres, equivalent to 55,217 waves to the inch; No. 38aM at about 4900 tenth-metres, equivalent to 51,836 waves to the inch; No. 38 cuts out the red end of the spectrum to about 6200 tenth-metres, allowing the remainder of the colours to pass practically unaltered.

Some Specially Good Substances for the Polariscope

Platino-cyanide of magnesia, of barium, of thallium, of potassium, of yttrium, and of strontium. Tartaric and citric acids. Chlorate of potash. Borax, menthol, ammonia-phosphate, thymol, chrysammate of lead, cinchonine, ferro-cyanide of potassium, grape sugar, nitrate of potash, nitro-prusside of soda, oxalate of chromium, of potash, and of ammonia. Pyrogallic acid, salacine, santonine, sulphate of copper, of cadmium, of nickel, of potash, and of magnesia. Acetalinide, melted on the slip.

Crystalline Objects for use with Convergent Polarised Light

Aragonite, baryta, sugar, titania, calcspar, mica, nitre, topaz, nitrate of soda, and ferro-cyanide of potassium.

These crystals are difficult to prepare, and are best bought ready made. The former group are mostly dissolved in a little water or spirits of wine, a few drops (one or two) being placed on the slip and evaporated. Quite different results are often obtained by using concentrated solutions as compared with solutions quite dilute; and if specimens are dried quickly or dry spontaneously.

Two New Methods of Adjusting Tube-Length

Two new methods, proposed respectively by Commander Ainslie, F.R.A.S., R.N., Naval Instructor, Royal Naval College, Greenwich, in the *English Mechanic*, June 18th, July 2nd, and July 9th, 1915, and by Dr. Hartridge, Fellow of King's College, in Part II. of the June number of the *Journal of the Royal Microscopical Society*, page 119, will be found of great interest. We regret, owing to the limits of space, being unable to add a full account of either suggestion, for both communications are of considerable interest and of much practical importance.

Table of Natural Sines

°	0′	15′ = ¼°	30′ = ½°	45′ = ¾°	°	0′	15′ = ¼°	30′ = ½°	45′ = ¾°
0	·0000	·0044	·0087	·0131	46	·7193	·7224	·7254	·7284
1	·0175	·0218	·0262	·0305	47	·7314	·7343	·7373	·7402
2	·0349	·0393	·0436	·0480	48	·7431	·7461	·7490	·7518
3	·0523	·0567	·0610	·0654	49	·7547	·7576	·7604	·7632
4	·0698	·0741	·0785	·0828	50	·7660	·7688	·7716	·7744
5	·0872	·0915	·0958	·1002	51	·7771	·7799	·7826	·7853
6	·1045	·1089	·1132	·1175	52	·7880	·7907	·7934	·7960
7	·1219	·1262	·1305	·1349	53	·7986	·8013	·8039	·8064
8	·1392	·1435	·1478	·1521	54	·8090	·8116	·8141	·8166
9	·1564	·1607	·1650	·1693	55	·8192	·8216	·8241	·8266
10	·1736	·1779	·1822	·1865	56	·8290	·8315	·8339	·8363
11	·1908	·1951	·1994	·2036	57	·8387	·8410	·8434	·8457
12	·2079	·2122	·2164	·2207	58	·8480	·8504	·8526	·8549
13	·2250	·2292	·2334	·2377	59	·8572	·8594	·8616	·8638
14	·2419	·2462	·2504	·2546	60	·8660	·8682	·8704	·8725
15	·2588	·2630	·2672	·2714	61	·8746	·8767	·8788	·8809
16	·2756	·2798	·2840	·2882	62	·8829	·8850	·8870	·8890
17	·2924	·2965	·3007	·3049	63	·8910	·8930	·8949	·8969
18	·3090	·3132	·3173	·3214	64	·8988	·9007	·9026	·9045
19	·3256	·3297	·3338	·3379	65	·9063	·9081	·9100	·9118
20	·3420	·3461	·3502	·3543	66	·9135	·9153	·9171	·9188
21	·3584	·3624	·3665	·3706	67	·9205	·9222	·9239	·9255
22	·3746	·3786	·3827	·3867	68	·9272	·9288	·9304	·9320
23	·3907	·3947	·3987	·4027	69	·9336	·9351	·9367	·9382
24	·4067	·4107	·4147	·4187	70	·9397	·9412	·9426	·9441
25	·4226	·4266	·4305	·4344	71	·9455	·9469	·9483	·9497
26	·4384	·4423	·4462	·4501	72	·9511	·9524	·9537	·9550
27	·4540	·4579	·4617	·4656	73	·9563	·9576	·9588	·9600
28	·4695	·4733	·4772	·4810	74	·9613	·9625	·9636	·9648
29	·4848	·4886	·4924	·4962	75	·9659	·9670	·9681	·9692
30	·5000	·5038	·5075	·5113	76	·9703	·9713	·9724	·9734
31	·5150	·5188	·5225	·5262	77	·9744	·9753	·9763	·9772
32	·5299	·5336	·5373	·5410	78	·9781	·9790	·9799	·9808
33	·5446	·5483	·5519	·5556	79	·9816	·9825	·9833	·9840
34	·5592	·5628	·5664	·5700	80	·9848	·9856	·9863	·9870
35	·5736	·5771	·5807	·5842	81	·9877	·9884	·9890	·9897
36	·5878	·5913	·5948	·5983	82	·9903	·9909	·9914	·9920
37	·6018	·6053	·6088	·6122	83	·9925	·9931	·9936	·9941
38	·6157	·6191	·6225	·6259	84	·9945	·9950	·9954	·9958
39	·6293	·6327	·6361	·6394	85	·9962	·9966	·9969	·9973
40	·6428	·6461	·6494	·6528	86	·9976	·9979	·9981	·9984
41	·6561	·6593	·6626	·6659	87	·9986	·9988	·9990	·9992
42	·6691	·6724	·6756	·6788	88	·9994	·9995	·9997	·9998
43	·6820	·6852	·6884	·6915	89	·9998	·9999	1·0000	1·0000
44	·6947	·6978	·7009	·7040	90	1·0000			
45	·7071	·7102	·7133	·7163					

Note.—The sine of any given angle is the length of the perpendicular opposite the given angle in a right-angled triangle which contains the given angle divided by the length of the hypotenuse. The above table is constructed on the principle that the hypotenuse is always equal to unity, by which means the fraction is got rid of, as the denominator may be left out. Thus,

$$\text{Sin } 30° = \frac{\text{perpendicular}}{\text{hypotenuse}} = \frac{\frac{1}{2}}{1} = \cdot 5.$$

ADDRESS LIST

OF OPTICIANS (AND OTHERS) MENTIONED IN THIS BOOK

(The tube-length for which their objectives are corrected is mentioned in brackets)

Angus, H. F., 83, Wigmore Street, London, W.1.

Baker, Charles, 244, High Holborn, London, W.C.1. (160 mm.)

Bausch & Lomb (Agents), 37 and 38, Hatton Garden, London, E.C.1. (160 mm.)

Beck, R. & J., 68, Cornhill, London, E.C.3. (160 mm.)

Flatters & Garnet, 309, Oxford Road, Manchester.

Kodak, Ltd., Kingsway, London, W.C.2.

Korista, F., Via G. Revere, N. 2, Milan. (160 mm.)

Powell & Lealand, "Emsdale," Greenham Road, London, N.10. (250 and 160 mm.)

Rheinberg & Co., 57-60, Holborn Viaduct, London, E.C.1.

Spencer Lens Co., Buffalo, N.Y. (160 mm.)

Swift & Son, 81, Tottenham Court Road, London, W.1. (160 mm.)

Watson & Son, 313, High Holborn, London, W.C.1. (6, 8, and 10 inches.)

Wray, Ltd., Grove Road, Bromley, Kent.

Most of the above will correct their objectives for other tube-lengths within prescribed limits, to order.

The cover-glass selected is usually ·17 or ·18 mm. for all opticians.

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PLATE I.

Figs. 1 and 2.—These photographs illustrate Dr. Van Heurck's test. Although selected by him for quite a different purpose, this diatom (Pleurosigma balticum) is useful for determining the steadiness of a stand and its adjustments. Using a magnification of a thousand diameters, the little balls seen in the centre of each photograph are focussed for their "white effect," as shown in Fig. 1. If the stand or the table on which it rests be rapped reasonably hard, the balls should not turn black as shown in Fig. 2. See pages 49-55.

Fig. 3A.—A diatom photographed with an objective of too low an aperture —"empty" magnification, as the late Professor Abbe called it. The dots are scarcely visible. See page 137.

Fig. 3B.—The *same* diatom at the *same* magnification, but photographed with a combination of higher numerical aperture. The dots can now be seen distinctly rendered. See page 137.

Fig. 4A.—The Abbe Test-plate (the 3 by 1 slip upon which the cover rests not being shown). See pages 351 and 362.

Fig. 4B.—A portion of the plate enlarged. The lines used for testing purposes can only be faintly glimpsed stretching along the floor of the white strips. See page 351.

Fig. 5.—The six spectra seen around the "direct" beam when looking at the back lens of an objective of N.A. 1·40, and Pleurosigma on the stage. The outer portion of each spectral beam is seen to be fainter than the inner because the photographic emulsion is less sensitive to the red rays than to the blue. See page 194.

Fig. 6.—The "venue" is here shown to be changed to obtain "oblique light." Indications of the presence of other spectral beams are distinctly visible. See page 194 *et seq.*

[Figs. 3A and 3B have been kindly lent by the Scientific Press from the author's book *Photomicrography.*]

PLATE I.

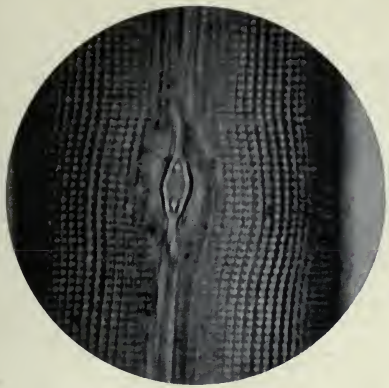

Fig. 1.

Fig. 2.

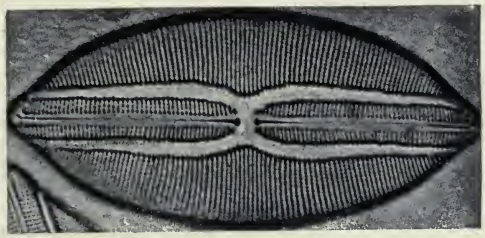

Fig. 3A.

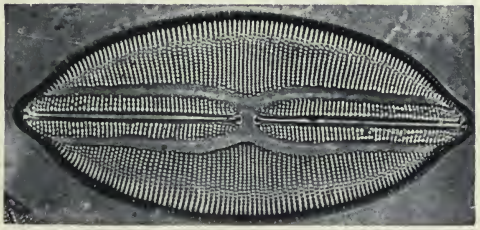

Fig. 3B.

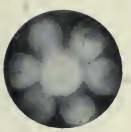

Fig. 5.

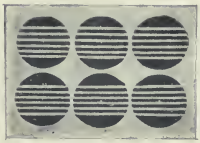

Fig. 4A.

Fig. 4B.

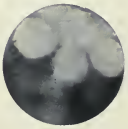

Fig. 6.

PLATE II.

Fig. 1.—Appearance presented by a first-class apochromat N.A. 1·40, 2 mm. focal length, with *direct white light* and Abbe test-plate. Edges of white line in *sharp* focus and definition perfect. See page 366.

Fig. 2.—Ditto, with *oblique white light.* There is scarcely any difference between the two images. See page 367.

Fig. 3.—*Under*-correction with *direct white light*; notice the falling off of definition, and the fluffiness at the edges of the white line. See page 366.

Fig. 4.—Ditto, with *oblique white light.* A curious wave of fluffiness distinctly visible, which in reality looks lifted up over the edge. See page 366.

PLATE II.

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

PLATE III.

Fig. 1.—Testing with Abbe plate continued from Plate II. *Over*-correction with *direct white light*, a marked fluffiness of both edges of white line and definition generally very poor and foggy. See page 366 *et seq.*

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Fig. 3.—Appearance presented by an objective (2 mm. apochromatic) in which the sine-law has not been fulfilled. *Outside* the focus the markings are seen to be *circularly* disposed. See page 369 *et seq.*

PLATE III.

Fig. 1.

Fig. 2.

Fig. 3 — Outside the Focus.

PLATE IV.

Fig. 1.—Testing with the Abbe plate continued from Plate III. Using the same combination as employed in Fig. 3, Plate III. this photograph depicts the appearance presented *within* the focus when using a combination in which the sine-law has not been fulfilled. The markings seem now to be arranged *radially*. See page 369 *et seq.*

Fig. 2.—AMPHIPLEURA PELLUCIDA. The lines in this particular specimen are about $\frac{1}{11\,0\,0\,0\,0}$ of an inch apart. With a good objective they should appear *extremely* fine.

Photographed with a Zeiss 2-mm. apochromat N.A. 1·30 × 1750. See page 195.

Fig. 3.—COSCINODISCUS OMPHALANTHUS. The central portion of the diatom is shown in the *centre of the photograph*, and the arrangement of the specimen is such that one focus of the object is seen on the right hand and the other on the left. It may be compared with Fig. 1, Plate VIII.

Photographed with a Reichert 2-mm. apochromat N.A. 1·35 × 750. See page 383.

PLATE IV.

Fig. 1.
Within the Focus.

Fig. 2.

Fig. 3.

PLATE V.

NAVICULA LYRA. This is a favourite specimen to examine the colour-correction of a semi-apochromatic objective. When well made and taken at the moment of focus, but little colour of the secondary spectrum should be visible; but the diatom appears tinted according to the colour having the shortest focal length: apple-green is that mostly chosen. With an apochromat this tinting should be entirely lost, and the valve ought to look the purest white, whilst the dots are colourless also. With those apochromats that exhibit the tertiary spectrum (see text, pages 380 and 393) the dots *may* show a *little* colour, but it is open to question whether this type of combination is not more perfect than that in which such exhibition is entirely absent. To test its superiority in definition monochromatic illumination is necessary. No fuzziness *whatever* should be present, and the dots ought to appear very neatly defined and crisply rendered.

Photographed with a Zeiss 3-mm. apochromat N.A. 1·40 × 500 and subsequently enlarged × 2. See pages 380 and 393.

PLATE V.

PLATE VI.

Fig. 1.—AMPHIPLEURA PELLUCIDA. In dots of about $\frac{1}{100,000}$ of an inch diameter. These are very difficult to see without the use of oblique green light, even when employing the finest objective. A first-class semi-apochromat should then show the dots furnishing an image *almost* as good as that afforded by the apochromat, although a certain distinction can in most cases be recognised, and the illumination is certainly less than with the higher corrected combination.

This photograph was taken (using blue light) with a Zeiss 2-mm. apochromat N.A. 1·40 × 4000. See pages 196 and 380.

Fig. 2.—PLEUROSIGMA ANGULATUM; the white-hexagon focus. In some parts where the upper layer of the diatom is denuded, the "black-dot effect" can be seen. Postage-stamp fracture is visible in the little lozenge and elsewhere at different places. The image with a good objective of either variety ought to be clean, sharp and clear, with but a very moderate closing of the substage iris diaphragm. See page 381.

Photographed with a Leitz 2-mm. apochromat N.A. 1·30 × 2000.

Fig. 3.—PLEUROSIGMA ANGULATUM showing in the *centre* of the photograph minute (apparent) apertures surrounded by a limiting boundary which is *semi-transparent and not so black as represented in the previous picture.* It is open to question whether this is not really the correct focus of this difficult diatom. The tube-length may require attention, as the perfectness of rendering and the cleanness of the image (its freedom from fog and greyness) are the real use of this test. Poor combinations will not furnish an image anything approaching the beauty shown in this photograph. See page 381.

Taken with a Reichert 2-mm. apochromat N.A. 1·35 × 1000.

Fig. 3A.—PLEUROSIGMA ANGULATUM. A postage-stamp fracture and black dot appearances—a dark background effect. It is difficult to believe these black dots are not holes, which some have doubted.

Taken with a Zeiss 2 mm. apochromatic N.A. 1·40 × 1250 blue light. See page 383.

PLATE VI.

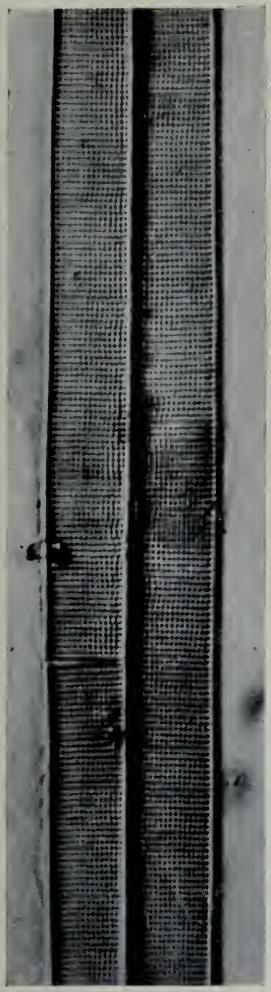

Fig. 1.

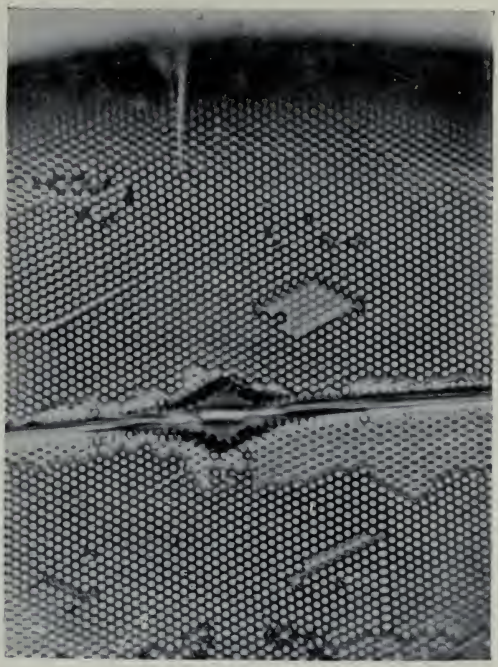

Fig. 2.

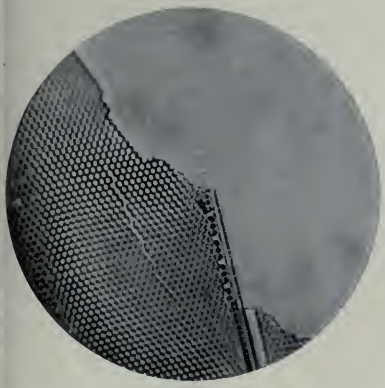

Fig. 3.

Fig 3A.

PLATE VII.

PLEUROSIGMA ANGULATUM. An enlargement of a photograph made with a 2-mm. apochromatic objective by Leitz of N.A. 1·35 to show the (probably) spurious markings which can be seen in the walls forming the hexagons when a certain focus is obtained. This photograph has not been taken with the aid of any specially shaped substage diaphragm or other arrangement, but merely in the ordinary manner at one particular focus.

We have usually noticed that to see these peculiar diffraction effects well shown the specimen must be mounted in realgar, and it is also necessary for the objective to be one of the finest computation and workmanship. If the components of the combination be not accurately centred or if the objective be poorly corrected, the image will not bear enlargement anything equal to the photograph as shown in this reproduction; about × 9000. See page 381.

PLATE VII.

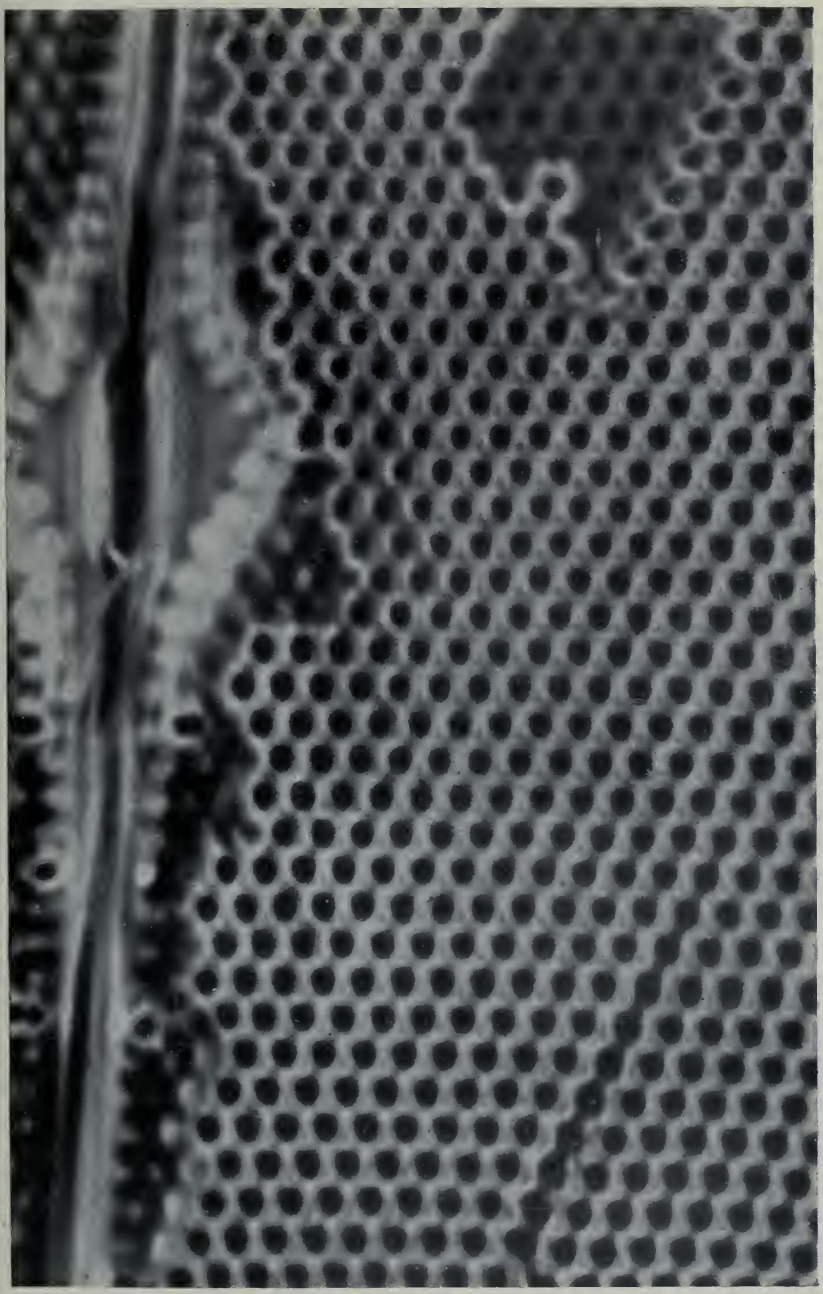

PLATE VIII.

Fig. 1.—COSCINODISCUS ASTEROMPHALUS—like the Ompthalanthus—has two distinct planes of focus, both being illustrated in Fig. 3, Plate IV. In this photograph, however, only one is shown, the situation selected being a point midway between the centre of the valve and its periphery, where specimens of this type are all for the most part flatter than elsewhere. See page 383.

Photographed with a Zeiss 3-mm. apochromat N.A. 1·40 × 1000.

Fig. 2.—PODURA SCALE. With a well-marked specimen a good apochromatic objective shows a constriction around the neck or upper part of the white interior of the "note," the white portion itself tapering off insensibly to a point at about the lower two-thirds of the note itself. If the objective be semi-apochromatic, much the same appearances should be present, especially if used with green light. Badly corrected combinations in the preferred colour even then render the white interior very fluffy as a rule, and indefinitely defined. See page 384.

Photographed with a Zeiss 3-mm. apochromat N.A. 1·40 × 1000.

Fig. 3.—A portion of Fig. 2 enlarged. See page 385.

Fig. 4.—SURIRELLA GEMMA. It is somewhat difficult to obtain a really flat specimen mounted in realgar. The dots should appear perfectly sharp and free from fuzziness at their edges. See page 384 and Fig. 2, Plate XVII.

Photographed with a Zeiss 3-mm. apochromat N.A. 1·40 × 3000.

Fig. 5.—FRUSTULE SAXONICA or VAN HEURCKIA CRASSINERVIS. The white dots are *exceedingly* minute. A good apochromat shows them well defined with oblique *white* light, and a semi-apochromat almost equally well with green illumination.

Photographed (green light) with a Zeiss 2-mm. apochromat N.A. 1·40 × 1200. See page 383.

[Figs. 1, 2, 3 and 4 have been kindly lent by the Scientific Press from the author's book *Photomicrography.*]

PLATE VIII.

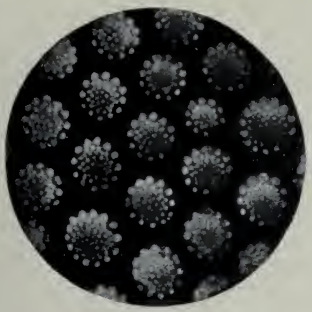

Fig. 1.

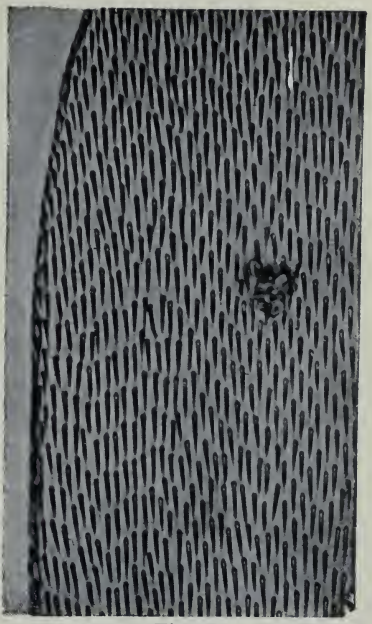

Fig. 2.

Fig. 3

Fig. 4.

Fig. 5.

PLATE IX.

Fig. 1.—NITZSCHIA OBTUSA. This diatom is a searching test for a good 2-mm. apochromat or a fine semi-apochromat of the same focal length. The striæ are fine, about 26 or 27 to the ·0003937 of an inch (Van Heurck), and there are about 15 dots in each. Although these can be *seen* with almost any reasonably good objective of the focal length mentioned with direct white light, still to show them sufficiently separated and discretely defined to permit of their being counted with ease requires the use of an objective that is well corrected and free from aberrations. Consequently a running together of the dots betokens a poor specimen of the optician's art. With an apochromat, the dots when focussed for the *white effect* should appear *distinctly* white—like pearls—and the background somewhat grey; but with a semi-apochromat they will be coloured by the secondary spectrum, which spoils their distinctness. With a green screen however they should be shown almost as distinct with either class of objective. The dots in the *black-focus effect* should look (with a good specimen) as if they had been *punched* out of black paper and laid upon the surface of the diatom. The apochromat must be expected to show them better than its rival when using white light, but with oblique green illumination the difference should not be anything like so striking. See page 385.

Photographed with a Reichert 2-mm. apochromat N.A. 1·35 × 1240.

Fig. 2.—BREBISSONIA BOECKII. This is a remarkably delicate test, the exceedingly small dots constituting the costæ being *very* difficult to resolve distinctly. With an apochromat and suitably adjusted oblique light (white) and a × 12 ocular they should be plainly visible in parts of the diatom, but with a semi-apochromat, unless it is a specially fine one, definition usually suffers more especially on account of the interfering effects of the secondary spectrum, although not from that cause *entirely*. With oblique green illumination the image produced by the cheaper type of combination should be vastly improved, but it usually even then is inferior to that furnished by the more highly corrected objective. This diatom offers, it can be understood, a good test to ascertain the fineness of the image in the *preferred* colour of any semi-apochromat. See page 385.

Photographed with a Leitz 2-mm. apochromat N.A. 1·35 × 1440.

Fig. 3.—SYNEDRA CRYSTALLINA. When using white light and × 18 ocular, to break up the transverse striæ into dots requires a well-corrected objective, with semi-apochromats especially. The black dots do not ever look so "punched out" as in the case of the Nitzschia obtusa, even with the best apochromats, unless green light be used, and even then they do not possess that marked circumscribed effect so particularly noticeable in that object. A poorly made semi-apochromat will be thrust out of its trials with this test-object, for the dots may appear as if surrounded by a thick fog. See page 385.

Photographed with a Koristka N.A. 1·5-mm. apochromat × 1200.

Fig. 4.—CYMBELLA GASTROIDES (small variety). There are not many diatoms that exhibit conical-shaped secondary markings such as can be seen in parts of this object. These, as well as others of differing form—some being more or less rectangular—should appear, when viewed with a first-class objective, as if "lifted out" from the background, which ought not to exhibit a trace of fluffiness or fog. See page 385.

Photographed with a Hartnack 2-mm. apochromat N.A. 1·40 × 1440.

PLATE IX.

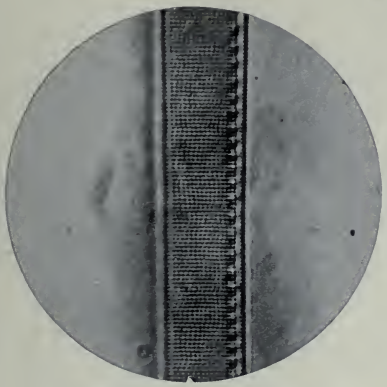

Fig. 1.

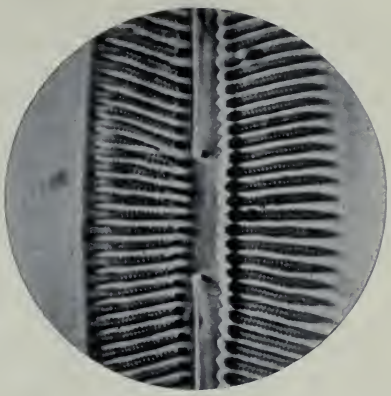

Fig. 2.

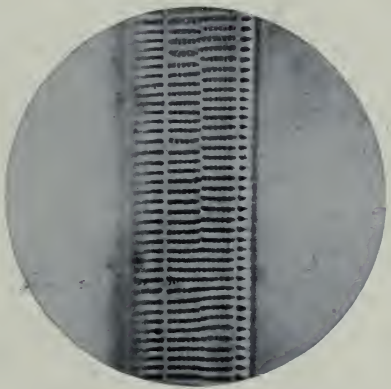

Fig. 3.

Fig. 4.

PLATE X.

NAVICULA SMITHII. The double row of circular dots in each costa should be well defined, anyhow in certain portions of the field of view. Van Heurck says: "The exact nature of these markings was not known until the introduction of the homogeneous objective." We have never been able to obtain an objective that will show the dots better than those exhibited in the photograph. A poor semi-apochromat—even with green light—gives an exceedingly foggy image, the dots being perhaps hardly visible. The test, though a severe one, is of a very reliable nature. As the valve is very saucer-shaped, it is impossible to photograph it in its entirety at one plane of focus.

Photographed with a Koristka 1·5-mm. apochromat N.A. 1·40 × 1200 and subsequently enlarged × 2. See page 386, and Plate XVI, Fig. 3.

PLATE X.

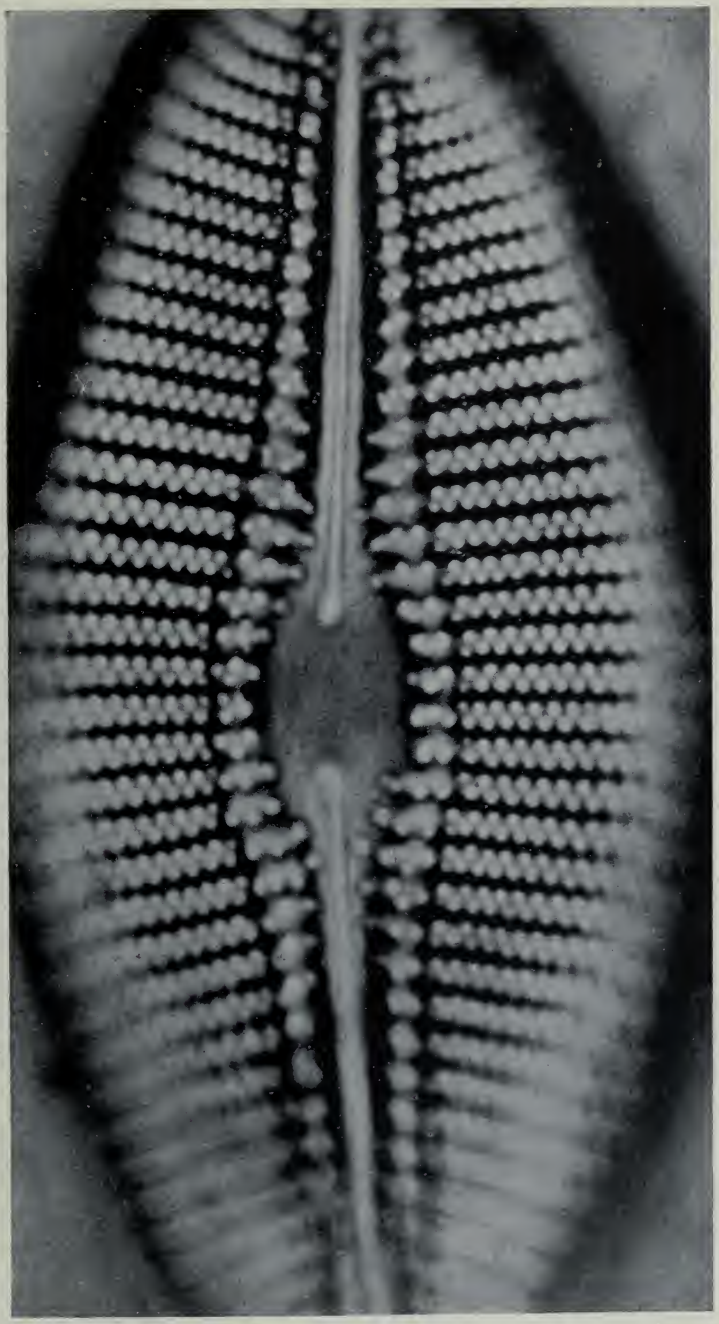

PLATE XI.

Fig. 1.—NAVICULA FIRMA. The exceedingly small dots in the floor of this diatom ought to appear discretely separated, free from all fuzziness and crisply defined. As the valve is not flat, both the white and the black dot "effects" are visible at the same focus in different parts of the specimen. See page 386.

Photographed with a Reichert 2-mm. apochromat N.A. 1·35 × 960 and subsequently enlarged × 2.

Fig. 2.—EPITHEMIA TURGIDA. The double row of irregular-shaped markings in this diatom, when magnified sufficiently, should appear well "lifted out" above the floor; and there should be no fluffiness of the background. The valve is so bent as to make it almost impossible to photograph satisfactorily, and it is not easy to be certain which is the correct focus, for at one plane the dots appear circular, at another hemispherical, whilst irregular-shaped ones also can be seen scattered about. The *cleanness* and *whiteness* of the entire object make it a good one to ascertain the type of the colour correction of a semi-apochromat, and the perfection of the correction in an apochromat. See page 386.

Photographed with a Leitz 2-mm. apochromat N.A. 1·35 × 1300.

PLATE XI.

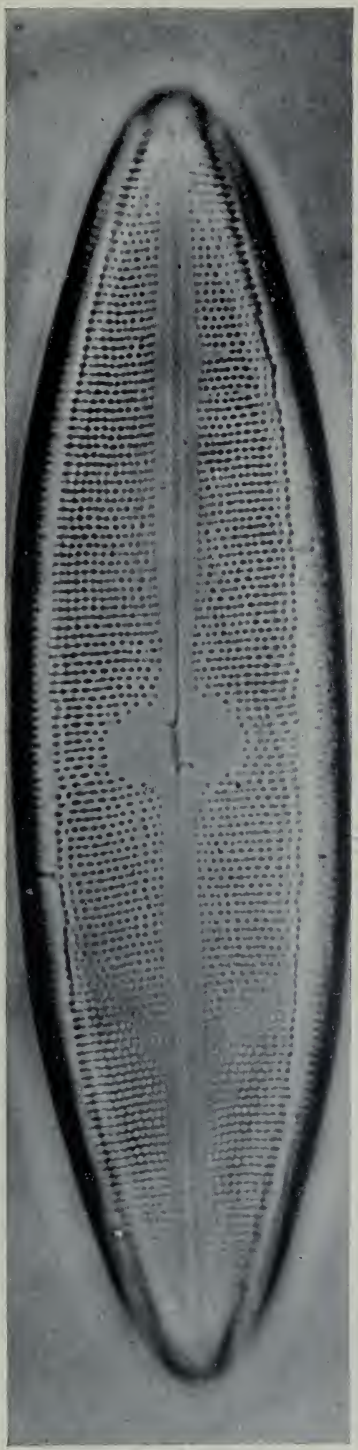

Fig. 1.

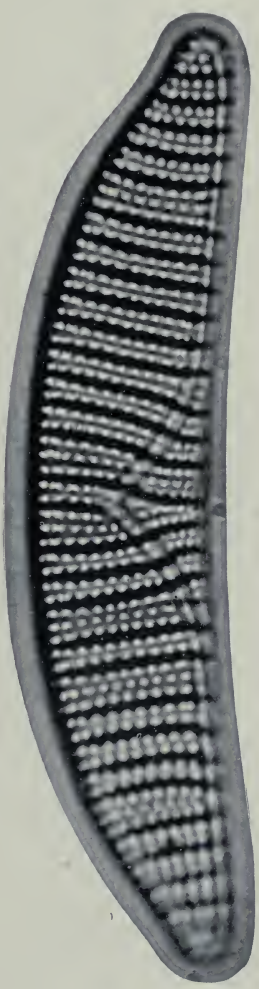

Fig. 2.

PLATE XII.

Fig. 1.—NAVICULA RHOMBOIDES. This is an old but still very favourite test-object with many opticians to test the type of colour-correction of a semi-apochromat, and also the perfection brought about in the computation and workmanship of an apochromat. The black-and-white dot "effects" are visible with this diatom by focussing the different planes. The dots at the back focus should be *intensely* black, and the whole diatom should look particularly brisk and bright, with an entire absence of all traces of fluffiness when using a well-made objective of high aperture. So too the white dots ought to appear like pearls laid upon the surface of the valve, no appearance of fog being visible. To obtain perfect definition with a semi-apochromat it may be necessary to use green light to remove the secondary spectrum. See page 387.

Photographed with a Zeiss 3-mm. apochromat N.A. 1·40 × 1200.

Fig. 2.—CYMATOPLEURA SOLEA. A most useful and delicate test-object. If looked at with a three-quarter cone and a × 12 ocular—direct white light—faintly marked transverse striæ should just be visible when employing a fine semi-apochromat or an apochromatic objective. Oblique white light reveals these striations—towards the median line particularly—as abruptly interrupted. Each striation should be so distinctly defined that it can be seen to terminate in rather a *round-shaped extremity*. An inferior combination will most likely fail to show the blunt ends, or perhaps may even fail to show the striations at all, the floor of the valve appearing a foggy desert void of detail. With a three-quarter cone (which is usually necessary) this object is a very searching test, and may enable the microscopist to differentiate between objectives which otherwise appear to perform equally well. See page 387.

Photographed with a Koristka 1·5-mm. apochromat N.A. 1·35 × 1200.

Fig. 3.—EUPLEURIA PULCHELLA (Arnot). This diatom is not frequently mentioned in the literature of the subject. When examined with direct white light (the outer zone being cut off by the iris substage diaphragm) it appears to be divided into about eighteen sections, most of which are quadrilateral, although a few are triangular in shape. Each section, if the upper surface be carefully focussed, presents numerous extremely minute dots crowded together. Even with full aperture the finest of apochromats will give faint though distinct indications of these dots. With oblique white illumination they should appear very distinctly separated with both types of objectives, green light of course rendering their presence far better. Second-rate combinations will show the dots, it is true, but they appear melted together and far from distinctly separated. Third-class objectives may fail to reveal their presence even with oblique green illumination. See page 387.

This diatom is also a very good test for ascertaining the quality of the definition in the outer part of the field of view. If the diatom be placed at the extreme edge of the field, and an objective be used in which the sine law has not been properly fulfilled, *no amount of focussing will bring the dots to a focus*. The quality of the definition affords the microscopist a good means of classifying objectives that otherwise perform very sensibly equal.

Photographed with a Hartnack 2-mm. apochromat N.A. 1·40 × 1000.

Fig. 4.—AMPHIPLEURA PELLUCIDA. To show the longitudinal lines with oblique green light suitably placed.

Photographed with a Zeiss 3 mm. apochromat × 1600. See page 196, footnote.

Fig. 5.—AMPHIPLEURA PELLUCIDA. With oblique *green* light suitably arranged to show the dots. Compare with 5 and 6, Plate XIII.

Taken with a Zeiss 3-mm. apochromat × 1400. See page 196.

PLATE XII.

Fig. 4.

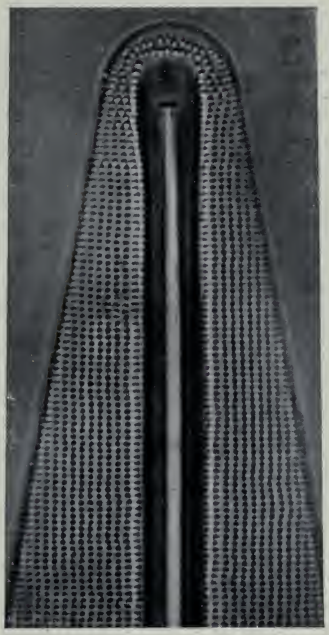

Fig. 1.

Fig. 5.

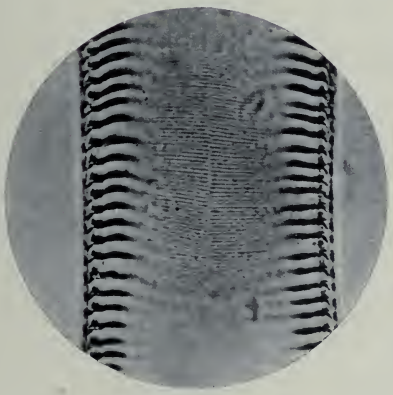

Fig. 2.

Fig. 3.

PLATE XIII.

Fig. 1.—NITZSCHIA SIGMA is a very small and exceedingly transparent diatom with minute dots. These should be completely and very distinctly resolved by a first-class 2-mm. objective with the iris diaphragm partially closed, a test more especially for central and medial zones.

Photographed with a Powell & Leland $\frac{1}{12}$th apochromat N.A. 1·40 × 1440. See page 387.

Fig. 2.—NITZSCHIA SCALARIS. This diatom is an exceedingly good test for a 4-mm. ($\frac{1}{6}$th) objective whether an apochromatic or semi-apochromatic combination. The dots should not only be *seen*, but they should be *distinctly separable*, provided the ocular be of sufficient power and the specimen a good one. No oblique light should be necessary, and (for the test to be critical) care must particularly be taken that the light from the mirror does *not* impinge with *any obliquity* upon the substage condenser.

Photographed with a Zeiss 4-mm. apochromat N.A. 0·95 × 800. See page 398.

Fig. 3—NITZSCHIA CURVULA. Another test-object for a 4-mm. almost as difficult as the preceding. The dots in this case are usually exceedingly faint, hence, if the combination be not well corrected, the resulting fluffiness may quite hide the secondary markings. See page 399.

Photographed with a Leitz 4-mm. apochromat N.A. 0·95 × 1000.

Fig. 4.—NITZSCHIA MAXIMA. Although the dots in this diatom are resolved fairly easily, a good objective is required to show them without the use of green light, as displayed in the accompanying photomicrograph. There should be an entire absence of all haze, which is readily visible if the correction of the combination be of a feeble character. See page 399.

Photographed with a Reichert 4-mm. apochromat N.A. 0·95 × 720.

Fig. 5.—AMPHIPLEURA PELLUCIDA. Oblique blue light (Zeiss blue glass). See page 196.

Photographed with a Leitz 2-mm. apochromat N.A. 1·30 × 1600.

Fig. 6.—AMPHIPLEURA PELLUCIDA. Oblique blue light (Zeiss blue glass).

Photographed with a Reichert 2-mm. apochromat N.A. 1·30 × 1500. See page 196.

Fig. 1.

Fig. 2.

Fig. 3.

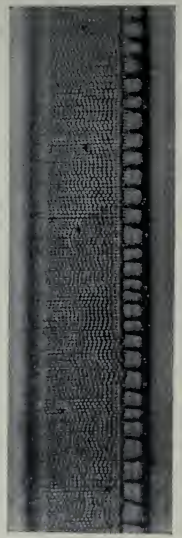

Fig. 4.

PLATE XIII.

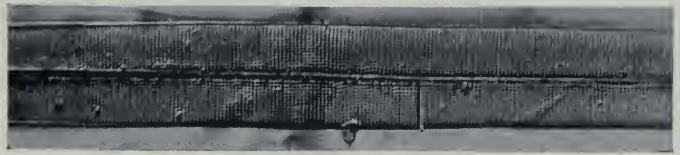

Fig. 5.

Fig. 6.

PLATE XIV.

Fig. 1.—PLEUROSIGMA ANGULATUM: the "black-dot" appearance. To show this focus well in a photograph with a 4-mm. is a severe test; the correction of the combination must be *very* good, and the better the objective the less the cutting down by the iris required. This is a point to be recollected. In the accompanying photograph the diaphragm was only closed a *very* small amount. See pages 381 and 396.

Photographed with a Koristka 4-mm. apochromat N.A. 0·95 × 1000.

Fig. 2.—NITZSCHIA OBTUSA. This diatom, before mentioned as a good test for a 2-mm., is also a very valuable though severe one for a $\frac{1}{3}$rd. Using oblique light it should be just possible to see the lines distinctly separated, if the combination be a fine one; but second-rate objectives will almost certainly fail in this respect. Occasionally, with a very well-marked specimen and a lucky green illumination, the dots, as shown in Fig. 1, Plate IX, may be firmly glimpsed; but the objective must be exceptionally well made. It is a most searching test, and will prove the superiority of one combination over another when many others fail. Owing however to its great transparency and the theoretical limit of resolving power being somewhat nearly reached, the valve must always be a well-marked one. See page 405.

Photographed with a Zeiss 8-mm. apochromat N.A. 0·95 × 360 and subsequently enlarged × 2.

Fig. 3.—STAURONEIS PHENICENTERON. This diatom is a good test object for a third of about ·65 numerical aperture. The dots are about $\frac{1}{33\frac{1}{3}\,0\,0}$ of an inch apart. These should be easily resolved, but very discretely and distinctly shown. A weak objective often covers them in fog. To show them as seen in the photograph it is best to use blue illumination × 700 with a Zeiss apochromatic 8-mm. apochromatic. See page 401.

PLATE XIV.

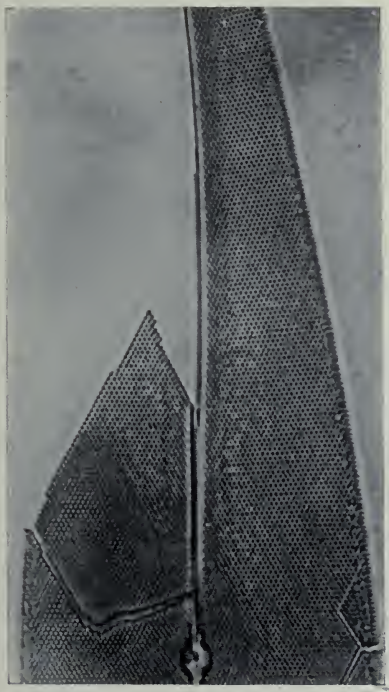

Fig. 1.

Fig. 2.

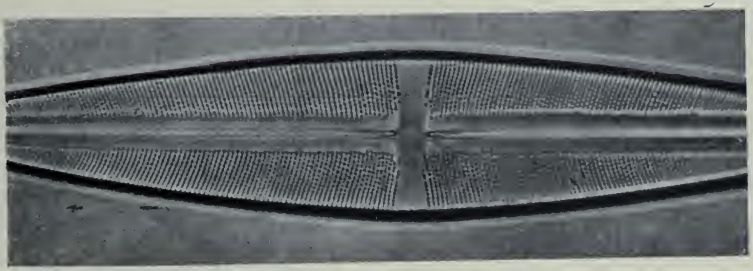

Fig. 3.

PLATE XV.

Fig. 1.—VAN HEURCKIA LOUISIANA. An excellent test-object for a 6-mm. objective, whether apochromatic or semi-apochromatic. Presuming the combination be one of high numerical aperture and used with a × 12 ocular, the faintest signs of secondary markings should be visible even at full aperture anyhow at the edges of the valve. With direct light and a small amount of cutting down with the iris substage diaphragm (not less than about ·7), the transverse striæ should be visible and well defined when the light is made oblique and green illumination employed (see photomicrograph). When the light is distinctly oblique and turned in the correct azimuth, the lines ought immediately to appear broken up into distinctly defined dots. A considerable difference of rendering will be noticed by different objectives, especially between the semi-apochromatic and the true apochromatic. The apochromatic by Zeiss with which the accompanying photomicrograph was taken (and subsequently enlarged twice) is perhaps the finest quarter it has been our privilege to test, but three semi-apochromatics approach it very nearly, notably those by Bausch & Lomb, Swift, and particularly the Holoscopic by Watson. See page 402.

Fig. 2.—AMPHIPLEURA PELLUCIDA in dots. Of the four photomicrographs of this diatom this is at the smallest magnification. It will be seen therefore that 1000 diameters is necessary to show the dots to anything like perfection.

Photographed (Zeiss blue glass) with a Koristka 1·5-mm. apochromat N.A. 1·35 × 1000. See pages 196 and 380.

Fig. 3.—ACTINOPTYCHUS BOLIVIENCIS. A rather difficult diatom to see with an inch. Photographed with a Zeiss 1-inch apochromat × 270. See page 406.

Fig. 4.—AMPHIPLEURA PELLUCIDA as seen with $\frac{1}{6}$ apochromatic oblique light and the Author's new blue screen mentioned in the Addenda. Details within the limits of possibility. Even dots can be seen in certain positions. Specimen about 110,000 lines to the inch. See page 402.

PLATE XV.

Fig. 1.

Fig. 2.

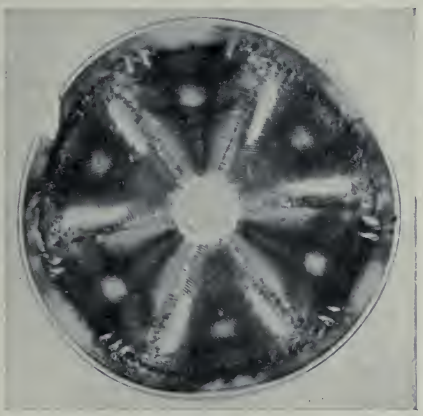

Fig. 3.

Fig. 4.

PLATE XVI.

Fig. 1.—PROBOSCIS OF THE BLOW-FLY. A common test for objectives of low power such as a 1 or 2 in. The best combination shows the object crisply defined, without a trace of fog, especially if the iris be closed a very small amount. The "blacks" should look *exceedingly* black, and not as if dusted over with a fine white powder or covered with a fog. No halo about the tracheæ should be observable. If the magnification be sufficient, the minutest hairs between the lobes should "rise up" as if growing from parts beneath. When properly illuminated no double tips to the hairs ought to be seen, and the large hairs should not show with an apochromat anything but a trace of colour along their edges. Colour may be seen to *a limited amount* with the best semi-apochromat. This object is also a very good one for testing *the size and flatness of field* in a low-power objective, and the Frontispiece (taken with a Holoscopic 24-mm. semi-apochromatic N.A. ·24 and two green screens) illustrates the perfection arrived at in a modern combination. See page 406.

Photographed with a Zeiss 24-mm. apochromat N.A. 0·3 × 60.

Fig. 2.—HEMIDISCUS CUNEIFORMIS. A very difficult diatom to resolve. Green light is better than white, but blue illumination is far superior. Zeiss 1-in. apochromat × 270. See page 407.

Fig. 3.—NAVICULA SMITHII. This is by far the *most* difficult to resolve into dots of any of the previous test objects for an inch. Slight oblique ligh: (by shifting the mirror) must be used, and it is best to have blue illumination. Zeiss apochromat 1-in. × 270. See page 407.

[Fig. 1 has been kindly lent by the Scientific Press from the author's book *Photomicrography.*]

PLATE XVI.

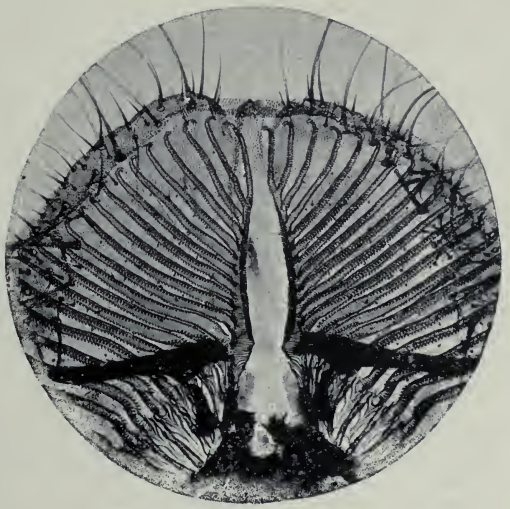

Fig. 1.

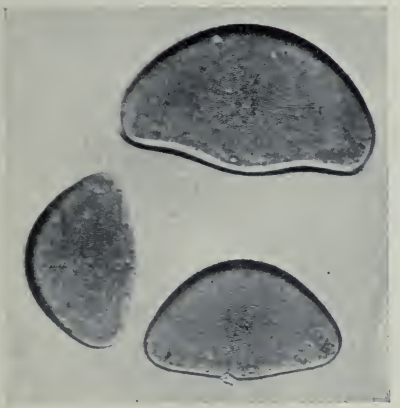

Fig. 2.

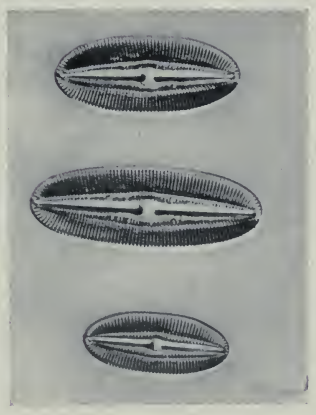

Fig. 3.

PLATE XVII.

Fig. 1.—PINNULARIA NOBILIS. An instruction photograph to those about to learn the use of moderately high powers. Photographed with a Zeiss $\frac{1}{6}$ apochromatic N.A. ·95. See page 371.

Fig. 2.—SURIRELLA GEMMA. An instruction photograph to those about to learn the use of high powers. Photographed with a Zeiss $\frac{1}{12}$ N.A. 1·40. See page 372.

PLATE XVII.

Fig. 1.

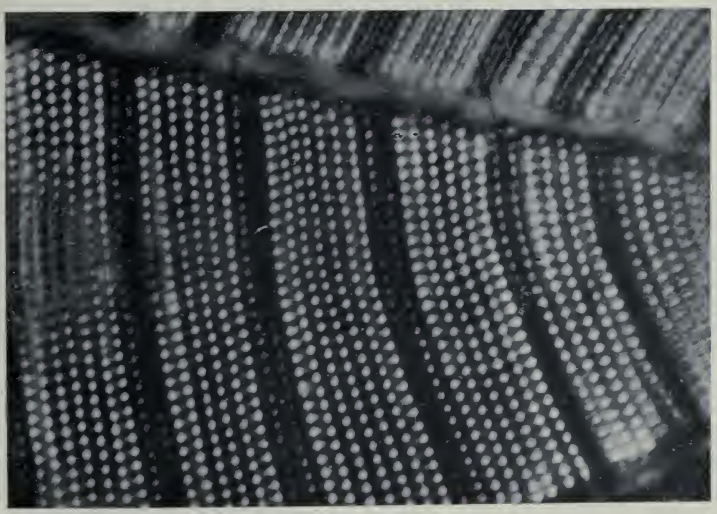

Fig. 2.

PLATE XVIII.

Fig. 1.—PYGIDIUM OF THE FLEA. On lowering the objective these long hairs come into view *first*, thus showing the specimen is *mounted correctly*. The hairs are rather peculiar, for they appear to have parallel sides until nearing their ends, when they suddenly taper off. Zeiss apochromatic $\frac{1}{6}$ N.A. ·95 × 340. See pages 389 and 400.

Fig. 2.—PYGIDIUM OF THE FLEA. The wheel discs with *completely* opened iris diaphragm. Not more fogginess than this should be present. Zeiss apochromatic $\frac{1}{6}$ N.A. ·95—full aperture × 820. See page 401.

Fig. 3.—PYGIDIUM OF THE FLEA. Wheel discs with $\frac{2}{3}$rds cone. Note the Maltese cross effects. The central dot is the central hair. With a good objective these discs should be most pronounced and clean. Zeiss apochromatic $\frac{1}{6}$ N.A. ·95, $\frac{2}{3}$rds cone × 864. See page 401.

PLATE XVIII.

Fig. 1.

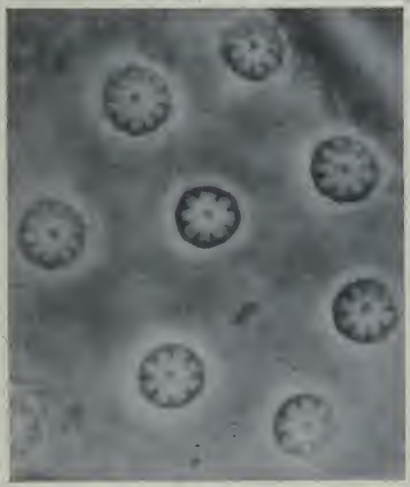

Fig. 2.

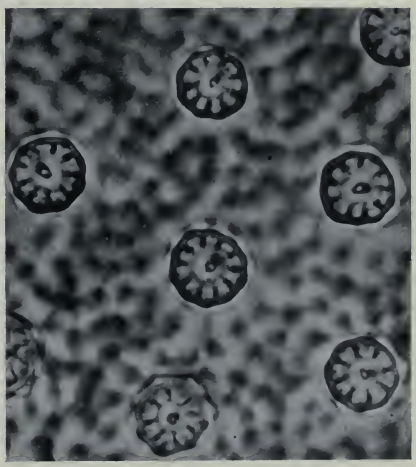

Fig. 3.

PLATE XIX.

Fig. 1.—PYGIDIUM OF THE FLEA. The hair-like structure as shown by a sixth. Zeiss apochromatic $\frac{1}{6}$ N.A. ·95 × 468. See page 400.

Fig. 2.—Part of Fig. 1 more magnified × 600. See page 400.

Fig. 3.—AMPHIPLEURA PELLUCIDA photographed in red light. No lines are to be seen. See page 493.

Fig. 4.—The same diatom photographed in blue light. The lines are distinctly visible. See page 493.

PLATE XIX.

Fig. 1.

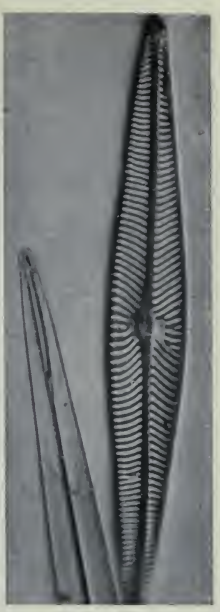

Fig. 3.

Fig. 2.

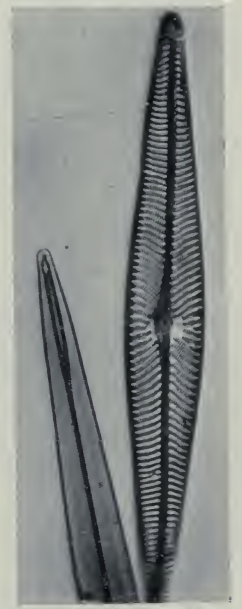

Fig. 4.

PLATE XX.

Fig. 1.—THE HEAD AND PROBOSCIS OF THE BLOW-FLY. Mounted without pressure. Photographed with a combination of 3½-inch focal length, reduced to F/16. To be compared with Fig. 2. See page 413.

Fig. 2.—The same specimen as Fig 1, but photographed with a combination of only 1½-inch focal length reduced to F/16. Notice the distinct improvement in the definition of the facets and the fine hairs in this photograph—taken with the lens of shorter focal length—as compared with that obtained with the combination of nearly twice the focal length. These two photographs, Fig. 1 and Fig. 2, are to show (see text) the greater depth of focus obtained by using lenses of short focal length over those of long focal length, the apertures being similar. See page 413.

Fig. 3.—PYGIDIUM OF THE FLEA. Low power view of the wheel discs. Zeiss $\frac{1}{6}$ apochromatic × 300. See page 401.

In the accompanying Plate (xx), the numbering of Figures 1 and 2 should be transposed

PLATE XX.

Fig. 1.

Fig. 2.

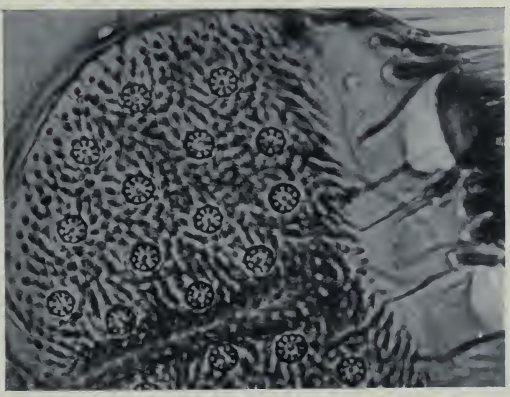

Fig. 3.

PLATE XXI.

Fig. 1.—NAVICULA CUSPIDA. This is a very difficult diatom to observe as well as to photograph with a sixth. Note the curious appearance of the white dots as explained in the text ; they are not really dots at all, but seem like mountain tops. See explanation to Plate XXIII. Zeiss $\frac{1}{6}$ apochromatic × 625, using blue light. (Specimen in Styrax.) See page 403.

Fig. 2.—SYNEDRA CAPITATA. A very difficult specimen to resolve with a $\frac{1}{12}$. Note the black dots (?) are elongated, being much longer with the length of the diatom than they are when transversely measured. Some are shown white and some dark, as this valve is supposed not to be quite flat. Zeiss apochromatic $\frac{1}{12}$ N.A. 1·40 × 1500. See page 388.

Fig. 3.—NAVICULA MAJOR. Most diatomists are aware that this specimen is very common, but few know that the tongues, if viewed with blue oblique light, will show linear markings of a very pronounced character. They are difficult to see. Zeiss apochromatic $\frac{1}{12}$ N.A. 1·40 × 2000. See page 392.

PLATE XXI.

Fig. 1.

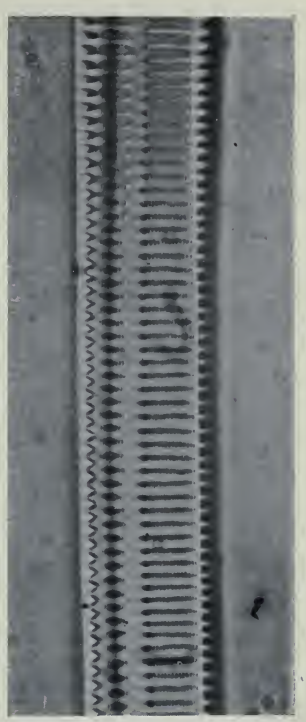

Fig. 2.

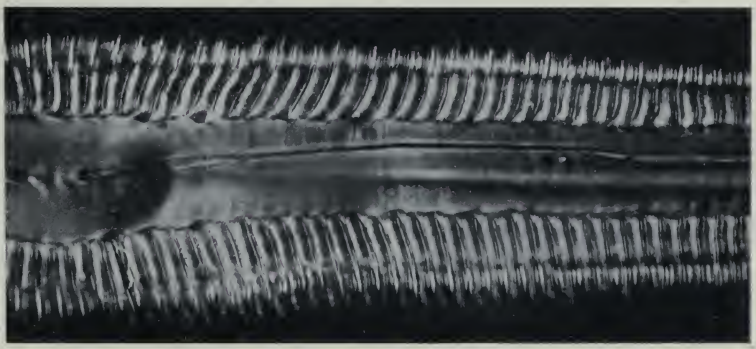

Fig. 3.

PLATE XXII.

Fig. 1.—NITZSCHIA SINGALENSIS. An enlargement of Fig. 3. Note how different the lines are in comparison with those of amphipleura, Fig. 2. These can be enlarged many times without showing any fuzziness. They seem to keep clean and neatly defined ; but not so with singalensis—they seem to merge off one into the other, so much so that they are apt to lose their individuality if enlarged much more than this photograph shows. About × 6000. See page 393.

Fig. 2.—AMPHIPLEURA PELLUCIDA. An enlargement of Fig. 2, Plate IV. About × 5536. See page 394.

Fig. 3.—NITZSCHIA SINGALENSIS. A diatom not much known. It is difficult to resolve unless the specimen be good. Most twelfth objectives will show the lines, but several do not pick them out as well as they might. Zeiss apochromat $\frac{1}{12}$ N.A. 1·40 × 2600. See page 393.

PLATE XXII.

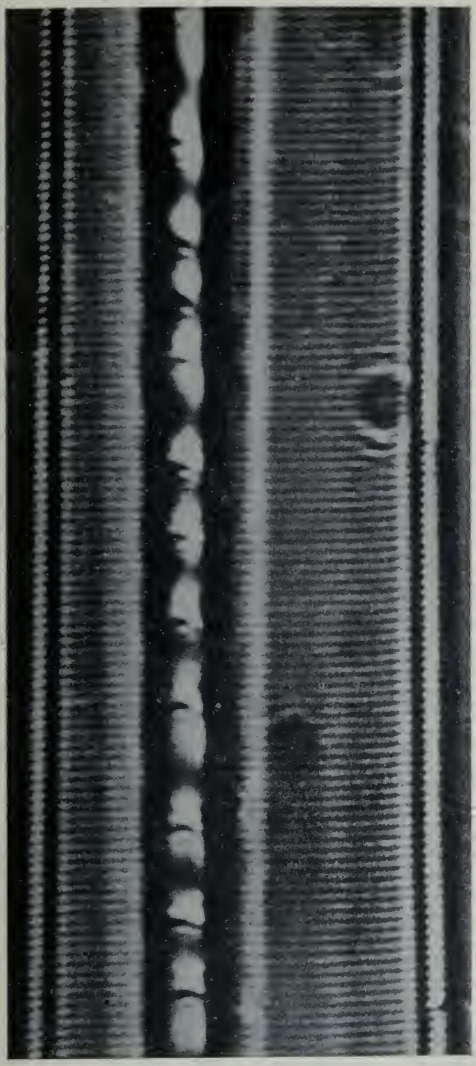

Fig. 1.

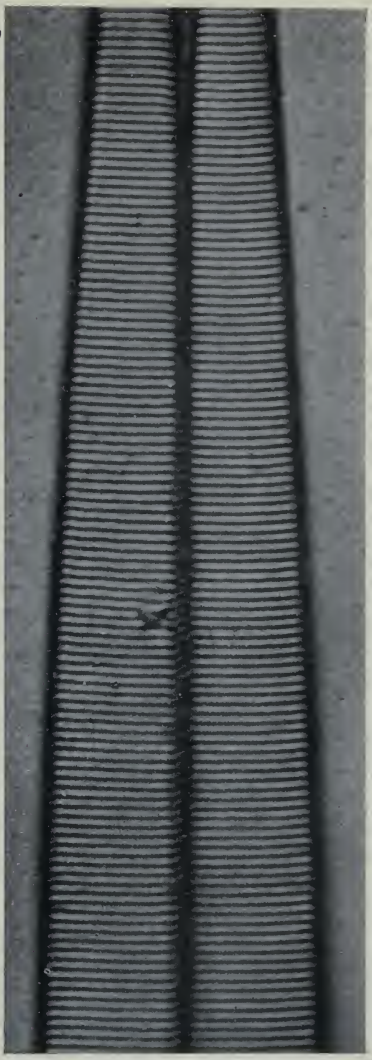

Fig 2.

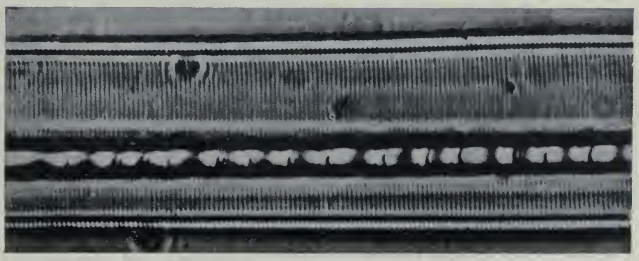

Fig. 3.

PLATE XXIII.

AN ENLARGEMENT OF FIG. 1, PLATE XXI, about × 1400. This peculiar diatom is worth studying carefully. The secondaries look as if they were real mountain elevations with shadow effects that make them stand out from their immediate surroundings. This shadow effect, however, is believed to be impossible with objects whose size is commensurate with a single wave-length of light. This is referred to in the text (which see). The white dots when magnified as in this plate do certainly appear as little mountains, but the black patches which alter their positions around the mountains according to the arrangement of the oblique light are most difficult to explain. At first the white dot may appear to become black with change of focus, but after a while it will be noticed this is not really the case.

Enlarged about $2\frac{1}{4}$ diameters × 1400. See page 404.

PLATE XXIII.

PLATE XXIV.

THE SOLDIER BEETLE. An example of an entire insect taken with the express object of showing the whole specimen in focus. Observe the claws, end of tail, jaws, and antennæ.

× 7. See page 414.

PLATE XXIV.

PLATE XXV.

THE FOOT OF AN AFRICAN SPIDER. To show how far such a class of specimen can be in focus though magnified × 12. Observe the definition of the claws and most parts of the solid portion of the leg. See pages 414 and 415.

PLATE XXV.

PLATE XXVI.

THE SPINEY SPIDER. A very difficult specimen (because not quite flat) to deal with, and to show how far it is possible to render the entire animal in focus at one and the same time. Note the extreme ends of the claws and the tips of the spines as well as the central portions of the body. × 12. See pages 414 and 415.

PLATE XXVI.

PLATE XXVII.

JUNCUS LAMPROCARPUS. A transverse section. Note how the whole of the tissues are in focus at one and the same time. × 30. See pages 414 and 415.

PLATE XXVII.

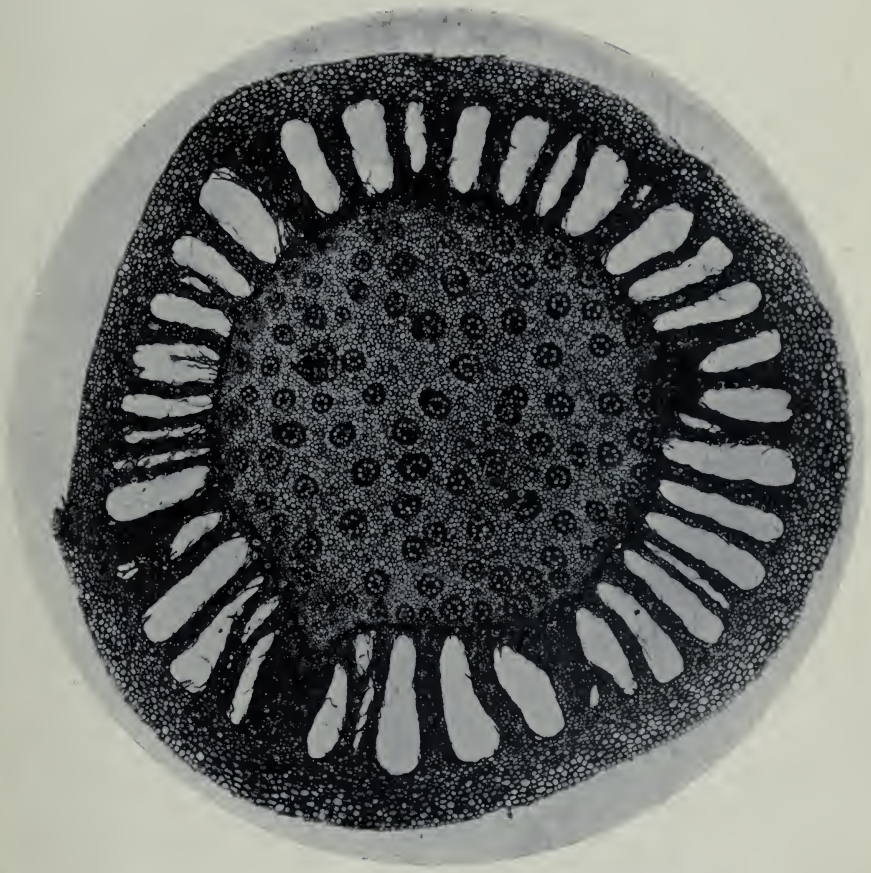

PLATE XXVIII.

Fig. 1.—PULEX IRRITANS. A most difficult specimen to show equally in focus all over. The body is thick and the back sausage-shaped, whilst the legs are so very long. Much patience is required to adapt the iris diaphragm, so that whilst obtaining sufficient depth of focus, definition is not spoilt by reducing the N.A. of the objectives. × 28. See pages 414 and 415.

Fig. 2.—THE FEMALE CORN-FLY. The difficulty in this specimen is not to lose the faint proboscis whilst focussing the body and extreme tips of the wings and legs. A badly corrected objective would play havoc with these details. × 8. See pages 414 and 415.

PLATE XXVIII.

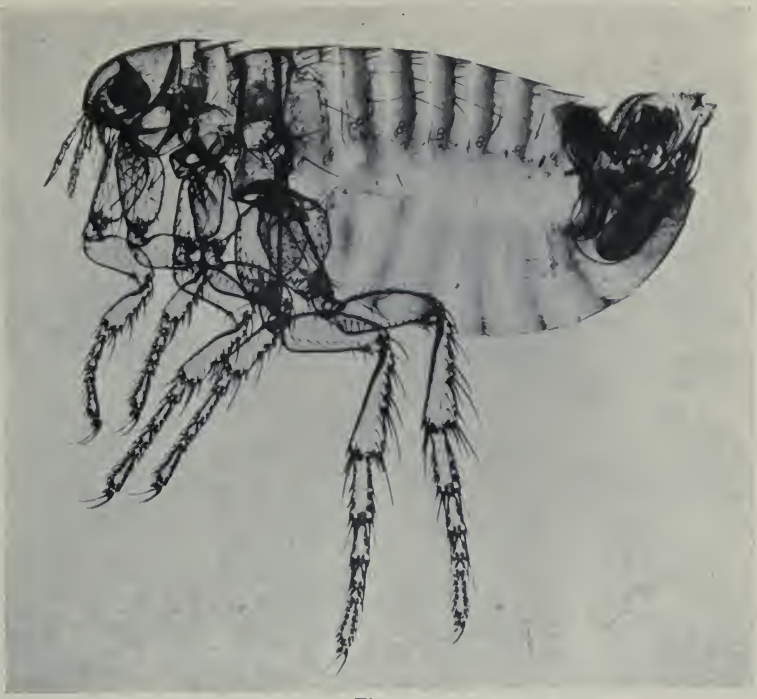

Fig. 1.

Fig. 2.

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